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Office of the Editor
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Department of the Environment
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Journal of the Fisheries Research Board of Canada

The Effects of Starvation and Subsequent Feeding on Survival and Growth of Fulton Channel Sockeye Salmon Fry (*Oncorhynchus nerka*)

H. T. Bilton and G. L. Robins

Fisheries Research Board of Canada
Pacific Biological Station, Nanaimo, B.C.

Bilton, H. T., and G. L. Robins. 1973. The effects of starvation and subsequent feeding on survival and growth of Fulton channel sockeye salmon fry (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 30: 1–5.

In the laboratory 8 groups of Fulton River channel fry were starved for periods ranging from 1 to 7 weeks, then offered food ad libitum for 8 weeks. Fry withstood up to 4-weeks starvation with less than 10% mortality; beyond this point mortality increased sharply to 90% at 7-weeks starvation. At 5-weeks starvation both fry length and weight decreased significantly. Mortality continued when fish were offered food ad libitum after being starved. Among fish starved for 1 and 2 weeks, mortalities continued to be low, 0 and 6.6%, respectively; but, among fish starved from 3 to 5 weeks, mortalities rose considerably, ranging from 42 to 99%.

Bilton, H. T., and G. L. Robins. 1973. The effects of starvation and subsequent feeding on survival and growth of Fulton channel sockeye salmon fry (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 30: 1–5.

Nous avons soumis en laboratoire 8 groupes d'alevins de saumon nerka du chenal de la rivière Fulton à un jeûne de 1 à 7 semaines, pour ensuite leur offrir de la nourriture *ad libitum* durant une période de 8 semaines. Les alevins subissent un jeûne de 4 semaines avec des mortalités de moins de 10%; passé cette période, les mortalités augmentent brusquement, et atteignent 90% après 7 semaines de jeûne. Après 5 semaines de jeûne, la longueur et le poids des alevins diminuent de façon significative. Les mortalités continuent lorsque, après leur jeûne, les poissons ont accès à de la nourriture *ad libitum*. Chez les poissons qui ont jeûné durant 1 à 2 semaines, les mortalités continuent à un bas niveau, 0 et 6.6% respectivement; par contre, chez les poissons qui ont jeûné durant 3 à 5 semaines, les mortalités augmentent considérablement sur une gamme de 42 à 99%.

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BABINE Lake, in northern British Columbia, produces approximately 90% of the sockeye salmon (*Oncorhynchus nerka*) from the Skeena River system (Larkin and McDonald 1968). Studies of the lake indicate, however, that it may be capable of supporting a larger number of young sockeye than at present (Johnson 1956). In 1965 an artificial spawning channel was completed on the Fulton River (one of the major spawning streams tributary to the lake) to augment the number of sockeye fry entering the lake. In 1969 a second spawning channel adjacent to the Fulton River was partially completed. Temperature data during its first winter of operation showed the channel to be warmer than that of the adjacent river (Paine 1971). As sockeye eggs incubated in the warmer channel water are likely to hatch and emigrate earlier than river sockeye, fry from the channel might reach the lake at a time when food was scarce and starvation could cause mortality.

The present study conducted in the laboratory was, therefore, undertaken to measure the ability of the channel fry (which, prior to the experiment, had not received external food) to survive without food for different periods and also to survive and grow when food was offered subsequent to starvation.

Materials and Methods

Several thousand sockeye fry from the second Fulton River channel were collected during the last week in May 1971, transported by truck (about 27 hr) to the Rosewall Creek Fish Culture Facility on Vancouver Island, and placed in a 1.8 m fiberglass tank, where they were held for 3 days without food prior to the experiment.

On May 28, 25 fry were randomly selected to determine initial lengths and weights of the stock. They were 30.1 mm, SD 1.22 and 0.143 g, SD 0.017. On the same date 400 fish were randomly selected, divided into groups of 50, and placed in eight separate (37.8 liter) fiberglass tanks.

All tanks had false mesh bottoms and outside standpipes. Flows were held constant at 1.89 liters/min. Fluorescent lights supplemented natural light from 0800 to 1630 hr each day.

Fish in group 1 (the control group) were offered food ad libitum daily throughout the 12-week experiment. Those in group 2 were starved for 1 week and then offered food ad libitum daily for 8 weeks. Group 3 were starved for 2 weeks, then offered food ad libitum daily for 8 weeks, and so on, with the starvation period increasing to 7 weeks but the subsequent feeding period remaining constant at 8 weeks. Mean water temperature during the experiment was 7.9 C, SD 0.29.

Frozen brine shrimp was chosen as food since it is superior to commercial mash as a "starter diet" for sockeye fry (Robins and Bilton 1971).

Each week a random sample of 25 fish were removed from group 1, anesthetized with 2-Phenoxyethanol, weighed, measured, and returned to the tank.

Fish in each of the other seven groups were sampled twice. At the end of the starvation period 20 fish were removed, weighed, and measured. At the end of the feeding period, if sufficient numbers remained, they were weighed and measured again.

Results

DAILY FEEDING

Both the mean length and weight of fish in group 1 (control) increased significantly between May 28 and August 20 (for length $t = 25.82$ and weight $t = 23.52$, df = 48, $P < 0.05$) (Fig. 1). During this period there was only one mortality (2%) which occurred on the 60th day from the beginning of the experiment (Table 1).

STARVATION

There was a significant decrease in mean length and weight (for length $t = 2.72$ and weight $t = 3.05$, df = 23, $P < 0.05$) between group 2 starved

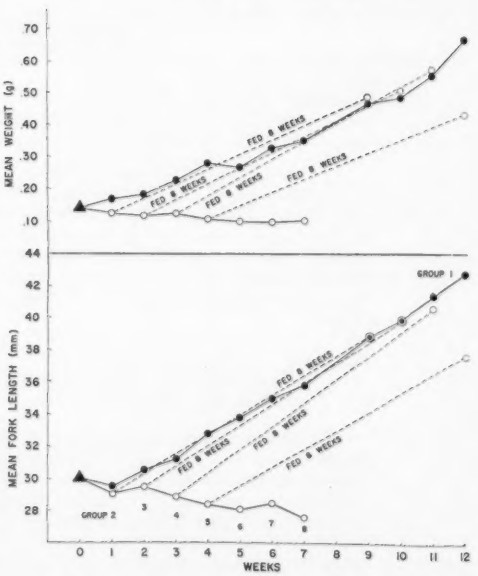

FIG. 1. Mean weights and fork lengths of fish in groups 2–8 (○) at the end of starvation periods ranging from 1 to 7 weeks and at the end of subsequent 8-week feeding periods. Also shown are the weekly means of fish in group 1 (●) fed throughout. ▲ represents the initial mean weight and length determined from 25 fry on May 28.

TABLE 1. Mortality within each group of sockeye fry during the period of starvation and subsequent feeding.

Group no.	Starvation period			Feeding period		
	No. fish	Weeks starved	Mortality %	No. fish	Weeks fed	Mortality %
1	–	–	–	50	12	2.0
2	50	1	0	30[a]	8	0
3	50	2	0	30[a]	8	6.6
4	50	3	2.0	29[a]	8	51.7
5	50	4	8.0	26[a]	8	42.3
6	50	5	18.0	21[a]	7	99.0
7	50	6	58.0	1	–	–
8	50	7	90.0	0	–	–

[a]Twenty fish sampled for lipid and moisture content.

for 1 week and group 8 starved for 7 weeks (Fig. 1). Comparison of mean length and weight of fish in groups 2–8 from week to week indicated few significant differences, but comparison with group 1 (control) each week indicated that in all but one instance lengths and weights of groups 2–8 were significantly smaller than those of group 1 (Table 2).

TABLE 2. Student t values comparing means of length and weight of fish from each group with group 1 on the same date.

Comparison between	Length[a]	No.	Weight
Starvation			
Groups 1 & 2 (June 4)	<0.813	45	<6.962[b]
Groups 1 & 3 (June 11)	<2.915	45	<9.810
Groups 1 & 4 (June 18)	<5.365	45	<10.100
Groups 1 & 5 (June 25)	<11.270	45	<16.900
Groups 1 & 6 (July 2)	<12.637	45	<15.744
Groups 1 & 7 (July 9)	<10.248	35	<5.126
Groups 1 & 8 (July 16)	<7.627	30	<9.688
Starvation and Feeding			
Groups 1 & 2 (July 30)	>0.048	45	>0.426
Groups 1 & 3 (Aug. 6)	>0.031	45	>0.888
Groups 1 & 4 (Aug. 13)	<1.003	39	<1.284
Groups 1 & 5 (Aug. 20)	<5.445	40	<5.819

[a]For example: Mean length of fish in Group 1 on June 11 was significantly larger than that of Group 3 on June 11.

[b]Underlined values indicate significance at 0.5% level.

Percent mortality increased with length of starvation period (Table 1). No mortalities occurred in those groups starved for 1 and 2 weeks. There was a highly significant positive correlation between mortality and weeks starved ($r = 0.881$, df = 5, $P < 0.01$). Substantial mortality (18%) first occurred among group 6 after 5-weeks starvation. These fish suffered a loss of 6.7% in length and 30.7% in weight, compared with the control group.

Mortality among starved fish began in groups 4 and 7 after 19 days (Fig. 2). A sudden increase in mortality occurred after 30 days. The pattern of die-offs among different groups was similar; the only difference being in total mortality. Comparisons (t-tests) of mean lengths of fish that had died from each group with the mean lengths of live fish

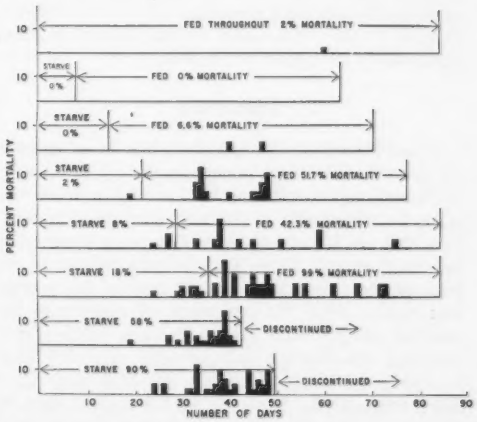

FIG. 2. Time of death and cumulative mortalities (%) for each group during periods of starvation and feeding.

from each of their respective groups at the end of the starvation periods showed no significant differences.

Starvation Followed by Feeding

In groups 7 and 8, 58 and 90%, respectively, of the fish died during starvation. Thus, the effects of feeding after starvation could only be measured among groups 2–6. Lengths and weights of groups 2–5, which had been starved for 1–4 weeks, increased significantly ($P<0.05$) after the 8-week feeding period (Fig. 1). A comparison of group 6 (starved for 5 weeks) could not be made as all but one fish had died by the end of 7 weeks.

Mean lengths and weights of fish in each of groups 2–4 were not significantly different ($P>0.05$) from those of group 1 (control) at the end of the feeding period (Table 2, Fig. 1). Fish in group 5 (starved for 4 weeks) were significantly smaller.

Fish continued to die off during the period of feeding following starvation in all but group 2 (Table 1). Among groups which had been previously starved from 2 to 5 weeks, however, mortalities ranged from 6.6 to 99% (Table 1). Percent mortality was greatest among groups which had been starved longer than 2 weeks.

Die-off of fish occurred up to 75 days after the beginning of the experiment (Fig. 2). The pattern of die-offs among groups was similar, but total mortality increased with starvation period. Comparison of mean lengths of fish that had died from each group during the subsequent feeding period with the mean lengths of live fish from each group at the end of starvation indicated that fish in group 4 (starved 3 weeks) and group 6 (starved 5 weeks) had grown significantly following starvation ($P<0.05$), whereas those in group 5 (starved 4 weeks) did not increase significantly in length ($P>0.05$).

Discussion

Under laboratory conditions Fulton River sockeye fry were capable of withstanding up to 3 or 4 weeks of starvation with less than 10% mortality. However, many were incapable of recovery later when presented with food. Beyond 4 weeks of starvation mortality increased sharply to 90% at 7-weeks starvation. In general, the pattern of die-offs among the groups was similar with the frequency of mortalities increasing sharply after about 30 days. Paine (1971) observed for starved channel and river fry that mortality occurred at means of 25.8 and 32.7 days, respectively. Thus, it would appear that in his experiment mortalities occurred earlier than in our experiment. This difference may have been associated with the water temperature. In our experiment the water originated from a well and thus the temperature was low and consistent. In Paine's experiment the water was drawn from the river and was subject to increasingly warmer temperatures (5.4–12.4 C) that could account for the earlier occurrence of mortalities.

Mortality continued when fish were offered food ad libitum after being starved. Among fish which had been starved for 1 and 2 weeks, mortalities continued to be low (0 and 6.6%, respectively). Among fish which had been starved from 3 to 5 weeks, however, mortalities rose considerably, ranging from a low of 42 to a high of 99%. These findings differ with those of Paine (1971), who observed that mortality increased sharply among channel fry submitted to 2-weeks starvation followed by 2-weeks feeding.

Both the length and weight of fry starved up to 7 weeks decreased significantly over the period. Results of a similar study for Fulton River fry (Paine 1971) also indicated that fry decreased in length over a 7-week period. A decrease in length was unexpected. However, Pickford (1953) observed that hypophysectomized mummichog (*Fundulus heteroclitus*) did not grow and sometimes actually decreased in length. The decrease in length of sockeye fry resulting from starvation may possibly result from resorption of cartilaginous material comprising the skeletal system (F. C. Withler personal communication).

Our results show that fish dying during starvation were of the same length as those which survived to the end of each starvation period. These findings tend to differ from those of Paine (1971), who found that Fulton River channel fry dying during a 3-week starvation period were significantly smaller than the survivors. There was no difference, however, between dead and live fry which had been starved 4 weeks.

An acceleration in growth rate was observed among some groups of fish which survived to the end of their 8-week feeding period after being starved. Those that had been previously starved from 1 to 3 weeks caught up in length and weight to the control group by the end of the experiment suggesting that these fry utilized food more efficiently. Such phenomena were demonstrated for trout whose diets had been increased (Brown 1946). Hence, it would appear that up to 3-weeks starvation does not prevent sockeye fry from attaining the size of others in the population that had not been starved. However, survivors after 4-weeks starvation and 8-weeks feeding had not caught up in either length or weight to the control group. This suggests that prolonged starvation inhibited their ability to fully utilize the food offered.

How might these findings bear on the survival of fry produced in the Fulton River spawning channel? Although the observed mortality is likely to be far greater than would be expected in Babine Lake because of the severe conditions under which fry were reared (sterile well water, physical disturbance, lack of cover, etc.), they could nevertheless be quite significant. It would appear that a high percentage of fry would be capable of surviving during and after 2-weeks starvation in the lake. However, even though most fry would also likely be capable of surviving a longer period of starvation of 3–4 weeks, a large mortality would likely occur later even after food became plentiful in the lake. Thus, it would appear that 2 weeks may be the maximum period of starvation that Fulton fry could withstand in the lake without suffering a significant mortality.

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Food of Larval Sea Lamprey (*Petromyzon marinus*) and American Brook Lamprey (*Lampetra lamottei*)

J. W. Moore and F. W. H. Beamish

Department of Zoology
University of Guelph, Guelph, Ont.

Moore, J. W., and F. W. H. Beamish. 1973. Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). J. Fish. Res. Board Can. 30: 7–15.

Throughout the year, algae were the most frequently encountered organisms in the gut of larvae of landlocked and anadromous sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). Relative abundance of organisms in the gut of both species was similar and did not change with length of larvae. In general, relative abundance of organisms in the gut was similar to that in samples of sediment and water. Occasionally, filamentous and episammic forms were not ingested because of their larger size.

The number of algal cells ingested per unit weight of sea lamprey decreased inversely with size of larvae. In the summer the number of algal cells in the gut was about thrice that found in the winter for larvae of comparable size. Survival among diatoms evacuated from the digestive tract varied from 45% in July (17.8 C) to 90% in January (0 C). Representatives of the *Achnanthes* had the lowest survival rate (10–20%), while those of *Amphora* and *Cyclotella* had the highest (65–100%). Complete evacuation required approximately 54 and 70 hr at 16.0 and 2.5 C, respectively. Evacuation rate did not differ among the organisms consumed. The numbers of algae consumed by larval sea lamprey in a stream relative to their estimated abundance was considered negligible.

Moore, J. W., and F. W. H. Beamish. 1973. Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). J. Fish. Res. Board Can. 30: 7–15.

Au cours de l'année, les algues sont les organismes les plus communément trouvés dans le canal alimentaire des ammocètes de la grande lamproie marine anadrome et landlockée (*Petromyzon marinus*) et de la lamproie de l'Est (*Lampetra lamottei*). L'abondance relative des organismes est la même dans le canal alimentaire des deux espèces, et ne varie pas avec la longueur de la larve. En général, l'abondance relative des organismes est la même dans le canal alimentaire et dans les échantillons de sédiment et d'eau. Occasionnellement, les formes filamenteuses et épipsammiques sont délaissées parce que trop grandes.

Le nombre de cellules algales absorbées par unité de poids par la grande lamproie marine diminue avec l'augmentation de la taille de l'ammocète. Le nombre de cellules dans le canal alimentaire est environ trois fois plus grand en été qu'en hiver chez des larves de taille comparable. La survie des diatomées évacuées par le tube digestif varie de 45% en juillet (17.8 C) à 90% en janvier (0 C). Le taux de survie le plus bas (10–20%) est celui d'*Achnanthes*, alors que le plus élevé (65–100%) se rencontre chez *Amphora* et *Cyclotella*. L'évacuation complète requiert environ 54 à 70 h à 16.0 et 2.5 C, respectivement. Le taux d'évacuation ne varie pas avec les organismes consommés. Le nombre d'algues absorbées par l'ammocète de la grande lamproie marine par rapport à l'abondance estimée des algues dans le cours d'eau est négligeable.

Received May 24, 1972

The diet of both parasitic and nonparasitic lampreys during the larval phase is reported to consist mainly of Aufwuchs, particularly, the diatoms. In the Upper Great Lakes, Manion (1967) found that larval sea lampreys (*Petromyzon marinus*) fed heavily on diatoms while Creaser and Hann (1929) observed both diatoms and desmids in the gut of American brook lampreys (*Lampetra lamottei*). Similarly, the diet of larvae of the European *Lampetra planeri* and *Eudontomyzon danfordi* (Schroll 1959) and the Mexican *Tetrapleurodon spadiceus* (Alvarez del Villar 1966) is mainly diatoms.

Food items are transported by the respiratory current through a ring of oral cirri into the pharynx

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TABLE 1. Rivers from which larvae of sea (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*) were collected.

Species	River	Basin	River length (*km*)	Station (*km* from mouth)	Date of sampling
P. marinus (landlocked)	Shelter Valley Creek	Lake Ontario	17	9–11	Dec. 1970–Nov. 1971 (monthly)
P. marinus (anadromous)	Pollett River	Bay of Fundy	50	40	Mar. 1971
	Little River		30	28	Sept. 1971
L. lamottei	Big Creek	Lake Erie	90	20	May, June, July, 1971

(Sterba 1962; Hardisty and Potter 1971). The oral cirri act as a sieve in preventing large particles from entering the pharyngeal chamber. Particles in the pharynx are agglutinated on a mucous band secreted by the endostyle and passed by cilia to the midgut. Selective agglutination has been reported by Schroll (1959) and Manion (1967).

This study was made to investigate the feeding biology of larva sea and American brook lampreys in streams tributary to the lower Great Lakes and anadromous sea lampreys in streams tributary to the Bay of Fundy. Particular attention was given to seasonal qualitative and quantitative aspects of availability, utilization, and digestion of food.

Materials and Methods

Ammocoetes were collected by electrofishing from the areas shown in Table 1 and separated into four length-classes; 0–3.5, 3.6–7.0, 7.1–10.5, and above 10.5 cm; irrespective of species or location. From the length frequency distribution for sea lamprey ammocoetes in Shelter Valley Creek, it was estimated that the size-classes 0–3.5, 3.6–7.0, and above 7.1 cm represent young-of-the-year, 1–2-year-olds, and 3–6-year-olds, respectively (Lowe 1972).

Each lamprey was preserved in Transeau's solution (Prescott 1962) immediately after capture. In the laboratory, contents of the anterior fourth of the midgut were examined and presumed representative of food items recently consumed. A wet mount of the gut contents was prepared for identification and enumeration of organisms other than diatoms at a magnification of 400×. Diatoms from the sample were cleared in concentrated nitric acid for 5 min, fixed in Hyrax mountant, enumerated, and identified at 1000×.

Three hundred organisms from the gut contents of each lamprey were identified (Table 2). In determining this sample size, 5000 organisms from the gut of each of several sea lampreys were counted on the assumption that the relative abundance thus obtained was in close agreement with the actual numbers present. From the same samples, relative abundance was then redetermined arbitrarily by examining 50, 100, 200, 300, and 500 organisms. Usually estimates of relative abundance from samples of 300 and 5000 organisms did not differ significantly ($P < 0.05$) using the chi-square test.

On two occasions, January and June 1971, the number of algal cells from the entire gut of sea lamprey ammocoetes collected at Shelter Valley Creek was estimated and the relative abundance of diatoms in the gut determined. After oxidizing the gut contents in 0.40 ml concentrated nitric acid, three 10-μliter aliquots were taken and all diatoms in each aliquot were counted.

Relative abundance of epipelic and planktonic organisms was estimated from samples of sediment and water, respectively (Table 2). Water samples were preserved in Transeau solution, passed through a Millipore filter (pore size, 0.45 μ) and a wet mount made from the filtrate. Epipelic organisms were identified and enumerated from a wet mount prepared from each preserved sediment sample.

Absolute abundance of algae in the plankton was estimated by counting all individuals in 10-ml water samples. Algal numbers in the sediment were estimated from a sample approximately 1 cm^2 in area and 2 mm in depth. The sample was dried at 55 C for 48 hr and weighed. Three subsamples were taken, weighed, and all individuals in each subsample were counted.

Diatoms were identified according to Hustedt (1930), Cleve-Euler (1951–55), and Patrick and Reimer (1966). Other algae were identified using Huber-Pestalozzi (1942–64) and Prescott (1962).

Only those taxa representing more than 1.0% of the number found in samples of sediment, water, and/or

TABLE 2. Sampling procedure employed in estimating relative abundance of organisms in gut contents and in river sediment and water.

	Ammocoetes	Sediment	Water
Sample no.	9–15/ length-class	40	6
Collection site	anterior midgut	upper 3 mm	2 cm off bottom
Sample volume	–	0.2 cm^3	90 ml
Organisms counted/sample	300	300	2000

gut contents are presented in this report. This technique accounted for 81.2–96.4% of the organisms found in the gut and 74.4–97.3% in the water and sediment. A complete listing of algae from the gut contents of ammocoetes, sediment, and water and their relative abundance for each collection made from the four rivers is available from the Depository of the National Research Council of Canada.

Feeding studies were made in the laboratory on sea lamprey ammocoetes maintained in floristically rich sediments and supplied with running nonchlorinated well water (10 ± 1 C). Light intensity at the sediment surface was 11 × 10^3 lux and photoperiod was 18 hr. Relative efficiency of the mucous band in agglutinating different species of algae was determined for 20 ammocoetes, 7–11 cm in length. A mixture of Chlorophyta (*Chlorella* sp. and *Scenedesmus bijuga*) and Bacillariophyta (*Achnanthes minutissima*, *Amphora ovalis* var. *pediculus*, *Gomphonema gracile*, and *G. olivaceum* and *Synedra ulna*) was released at the entrance of each feeding burrow. After 5 min, the pharyngeal chamber and midgut were removed and the contents identified and counted.

Rate of evacuation was examined for 20 ammocoetes, 7–10 cm in length, at each of 16.0 ± 1.0 and 2.5 ± 0.5 C. Larvae were held at their acclimation temperature for 28 days and allowed to feed on various unidentified species of shelled and ciliated protozoans, rotifers, and those members of the Chlorophyta and Bacillariophyta cited above. Thereafter, the ammocoetes were removed, placed in a gravel substrate without organisms, and the gut contents were examined at intervals until empty.

Availability of Food

Algae, which formed the main component in the gut of larvae of both the anadromous and landlocked sea lamprey as well as the American brook lamprey, accounted for 98.6–100% of the microorganisms (by numbers) in both the sediment and water samples in all rivers. At Shelter Valley Creek and in general for Big Creek, Little River, and the Pollett River, the most abundant algae in the plankton were the same as those found in the sediment.

The seasonal succession of algae at Shelter Valley Creek was characterized by maximum relative abundance of most Chlorophyta, Euglenophyta, and Chrysophyceae during summer and maxima of Cyanophyta during the fall (Moore 1972). The season of maximum relative abundance for diatoms varied among species (Fig. 1A–D). For example, maximum abundance of *A. minutissima* occurred from June to September while that for *Navicula tripunctata* occurred from December to May. Some diatoms showed little seasonal change in relative abundance, examples being *A. ovalis* var. *pediculus*, *Navicula capitata*, *N. cryptocephala* and varieties, *N. odiosa*, and *Nitzschia dissipata* and varieties.

Absolute number of algae on the sediment at Shelter Valley Creek decreased from 4.1×10^5 to 2.1×10^5 cells/cm^2 between November 1970 and February 1971 and thereafter increased in March to 4.6×10^5 cells/cm^2 before again decreasing in April to 2.1×10^5 cells/cm^2. Numbers of algae increased precipitously to 1.36×10^6 cells/cm^2 in May and June. This increase continued to August when numbers reached 1.4×10^6 cells/cm^2, the annual maximum. During September and October algal numbers fell to approximately 8.0×10^5 cells/cm^2.

Seasonal fluctuations in the numbers of algae in the plankton corresponded closely to those of the sediments. Winter values ranged from 2.0–6.4 × 10^4 cells/liter. During April, numbers fell to an annual minimum of 5.0×10^3 cells/liter. The annual maximum number of cells, 1.8×10^5, was found during June.

Relative abundance of algae in the sediment and water of the Pollett River during March and of Big Creek during May is presented in Fig. 1E–F. As sampling was made infrequently, it was not possible to follow the seasonal succession of algae. However, in both rivers, as well as the Little River, diatoms formed the most abundant algal forms.

In all rivers the most frequently encountered organism exclusive of algae was the protozoan *Difflugia* Leclerc, irrespective of season. Usually *Difflugia* accounted for less than 0.2% by numbers of the organisms in samples of sediment and water.

Utilization and Selectivity of Diatoms

Relative abundance of most species of algae found in the gut of landlocked and anadromous sea lampreys and American brook lampreys was similar to that found in samples of sediment and water for a given season (Fig. 1). There were, however, a number of instances in which certain algae appeared in the gut with different frequency than in the sediment or water. Notably fewer were *A. minutissima* and *Cocconeis placentula* var. *euglypta* (Fig. 1A, C, E); more frequent were *Nitzschia acicularis* (Fig. 1B, D) and *N. subtilis* var. *paleacea* (Fig. 1C). Throughout much of the year, the diatoms *Navicula gregaria* and *N. cryptocephala* var. *veneta* appeared more frequently in the gut than in either the sediment or water (Fig. 2).

Generally, composition of gut contents for a given season and river did not change greatly among individuals of either species irrespective of larval length (Table 3, Fig. 1). Further, there was no consistent pattern of change in size of ingested algae with length of larvae. The longest algal species frequently found in both lampreys was *Nitzschia sigmoidea*. The length of *N. sigmoidea* in the gut was similar to that in the environment regardless of larval length. For example, mean length of *N.*

A SHELTER VALLEY CREEK, DECEMBER 1970
B SHELTER VALLEY CREEK, MAY 1971
C SHELTER VALLEY CREEK, AUGUST 1971
RELATIVE ABUNDANCE, %
Sediment
Water
Larvae (0–3.5 cm)
Larvae (3.6–7.0 cm)
Larvae (7.1–10.5 cm)
Larvae (above 10.5 cm)
Achnanthes lanceolata
A. minutissima
A. lanceolata v. dubia
Amphora ovalis v. pediculus
Cocconeis pediculus
C. placentula v. euglypta
C. placentula v. lineata
Cyclotella meneghiniana
Cymbella cesp/cistosa
Diatoma vulgare
Fragilaria construens
F. construens v. venter
Gomphonema olivaceum v. balticum
Meridion circulare
Navicula capitata
N. cryptocephala
N. cryptocephala v. veneta
N. decussis
N. gregaria
N. mengra
N. menisculus v. upsaliensis
N. odiosa
N. pupula
N. tripunctata
N. salinarum v. intermedia
N. virudula
Nitzschia dissipata
N. dissipata v. media
N. linearis
N. subtilis v. paleacea
Synedra ulna
all Chlorophyta
all Cyanophyta
all Euglenophyta

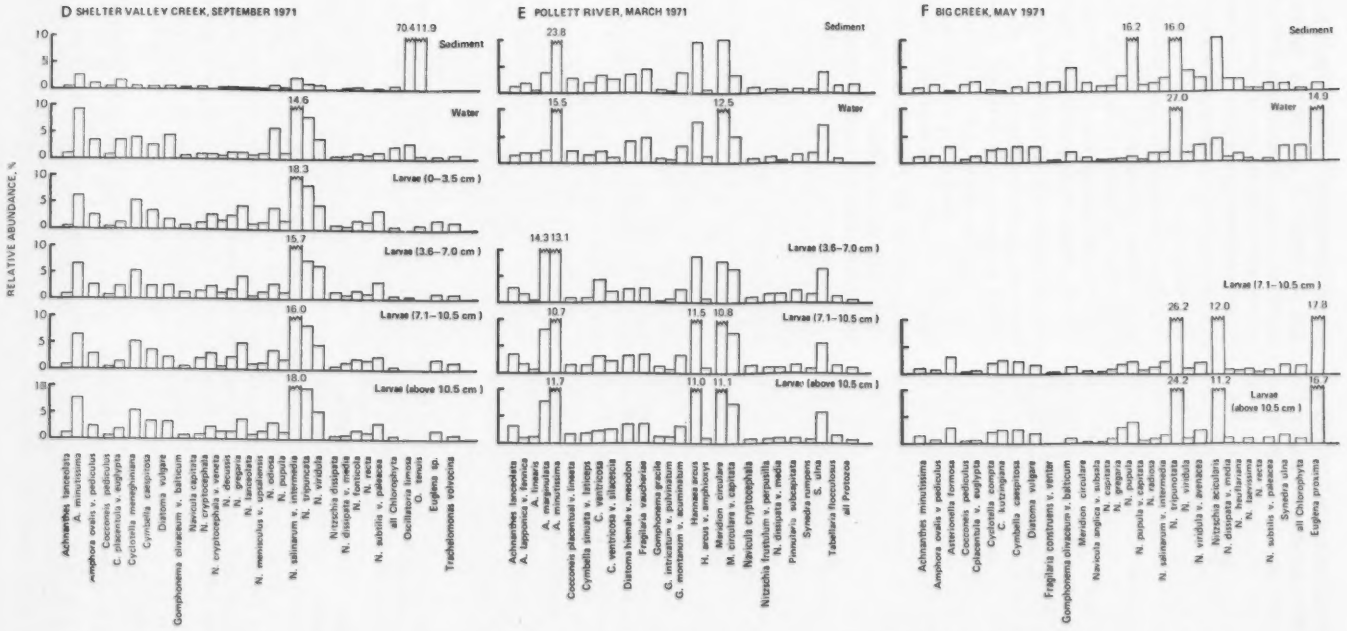

FIG. 1. Mean relative abundance of algae found in samples of sediment, water, and in the anterior portion of the midgut of lampreys of different size. Sea lamprey larvae were collected from Shelter Valley Creek, Ont., A–D, and the Pollett River, N.B., E, while American brook lampreys were captured from Big Creek, Ont., F.

sigmoidea in Shelter Valley Creek was 310 μ (range 260–400 μ). In the gut of sea lampreys, mean lengths of *N. sigmoidea* were: lampreys >10.5 cm, 313 μ (range 280–380 μ); 7.1–10.5 cm, 330 μ (range 280–380 μ); 3.6–7.0 cm, 310 μ (range 270–350 μ); and 0–3.5 cm, 340 μ (range 280–390 μ). Similar values were obtained for *N. sigmoidea* in the gut of American brook lampreys in Big Creek. Algae comparable in size to *N. sigmoidea* were not found in the other rivers.

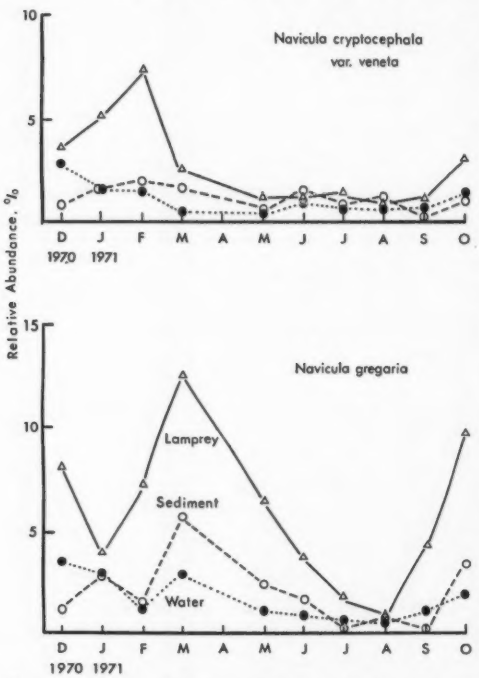

FIG. 2. Mean relative abundance of the diatoms *Navicula cryptocephala* var. *veneta* and *N. gregaria* from samples of the anterior portion of the midgut of sea lamprey larvae (all size-classes combined), sediment and water collected at Shelter Valley Creek, Ont.

The smallest algae frequently found in both lampreys were the diatoms *A. minutissima* and *A. ovalis* var. *pediculus*. Both had a mean length of 5 μ in the environment and gut. There was no indication that they were more abundant in the gut of larvae of one size-class than any other.

In contrast to observations on sea and American brook lampreys, Schroll (1959) found that the size and composition of diatoms in the gut of larval *L. planeri* and *E. danfordi* differed with age (length). Among yearling maximum particle size was 35 μ compared to 100 μ for 4-year-old larvae. However, these differences appear more related to changes in stream habitat preference with age than to changes in food selectivity. In those streams sampled in the present study there was no evidence to suggest that lamprey larvae of either species occupied different habitats with age. Rather, in areas of larval abundance all age-classes were invariably present.

Sand grains and detritus were frequently found in the gut of larvae, particularly in the winter in accord with the earlier observations of Schroll (1959). The mean diameter of sand grains from the gut, 30 μ, was considerably less than the longest dimension of most ingested algae.

Relative abundance of the diatoms *C. placentula* var. *euglypta* and *A. minutissima* in the gut of both species of lampreys was on several occasions lower than in samples of surface sediment and water. In addition, relative abundance of *A. ovalis* var.

TABLE 3. Mean (± 1 SE) expressed as percent by numbers of the dominant alga from the anterior portion of the midgut of sea lamprey larvae collected in Shelter Valley Creek in August (A) and September (S) 1971. Ranges are in parentheses; number of specimens examined was 10 for each size-class.

Sea lamprey (*cm*)	*Achnanthes minutissima*		*Cyclotella meneghiniana*		*Navicula salinarum* var. *intermedia*		*Nitzschia subtilis* var. *paleacea*	*Navicula tripunctata*
	A	S	A	S	A	S	A	S
0–3.5	8.3±1.2 (3.3–26.3)	6.2±0.7 (3.7–10.0)	5.0±1.2 (1.0–7.0)	5.2±0.6 (2.0–8.0)	19.9±1.9 (7.7–33.3)	18.3±0.9 (16.0–22.3)	18.7±2.3 (7.0–45.0)	8.2±2.0 (3.7–25.0)
3.6–7.0	10.8±2.3 (2.7–13.7)	6.7±0.7 (3.7–9.0)	3.8±0.6 (1.0–12.0)	5.4±0.6 (3.3–8.7)	20.9±2.3 (10.3–29.0)	15.7±0.7 (13.3–18.3)	17.7±4.5 (11.0–31.0)	7.3±0.8 (3.7–12.7)
7.1–10.5	11.0±1.7 (3.3–22.0)	6.6±0.8 (3.7–9.0)	5.4±0.5 (3.3–8.7)	5.2±0.5 (3.3–7.3)	23.5±2.0 (13.3–33.3)	16.0±1.1 (10.7–22.0)	11.4±1.7 (5.3–21.3)	8.1±0.8 (4.3–12.7)
>10.5	8.3±1.4 (2.7–16.7)	7.8±0.9 (4.3–12.7)	6.0±1.2 (1.0–11.0)	5.4±0.7 (3.0–10.7)	28.1±2.0 (16.7–38.0)	18.0±1.3 (13.3–25.3)	11.2±2.2 (4.7–25.0)	9.5±1.2 (3.3–12.3)

pediculus, *Achnanthes* spp. other than *A. minutissima*, and *Cocconeis* spp. other than *C. placentula* var. *euglypta* in the gut was generally lower, although not significantly, than in samples of sediment and water. Customarily *Achnanthes* spp., *Amphora*, and *Cocconeis* spp. were attached to sand grains 50–500 μ in diameter when they occurred in the sediments and water. Since sand grains in the gut averaged only 30 μ, scarcity of these taxa in the gut may be explained by an inability of larvae to transport particles of this size in the respiratory current.

During March at Shelter Valley Creek, relative abundance of *Diatoma vulgare* in the gut was lower than in either the water or sediment. At this time *D. vulgare* formed filaments up to 1-mm in length. Presumably filaments of this length are unable to pass through the oral cirri. Similarly, during September in Shelter Valley Creek, *Oscillatoria limosa* and *O. tenuis* were abundant on the surface of the sediments and common in the plankton, but virtually absent from the gut. Again, exclusion from the diet was attributed to size of algae.

Throughout the year at Shelter Valley Creek, several algal taxa, particularly the diatoms *N. cryptocephala* var. *veneta* and *N. gregaria*, occurred with greater relative frequency in the gut than in the environment. This suggests the larvae may have selected for particular kinds of algae, which is in accord with the discussions of Schroll (1959) and Manion (1967). In the present study, however, larvae were unable to ingest those algae above a critical size, such as the episammic genera *Achnanthes*, *Amphora*, and *Cocconeis*. Selection against these taxa implied all other algae must have appeared in the gut with greater relative abundance than in the environment. Hence, the apparent selection for certain algae is actually the result of exclusion of other algae because of their size. An example of this principle is illustrated in Fig. 1D where *O. limosa* and *O. tenuis*, the most abundant algal taxa on the sediments, were excluded from the gut because of their length. When the relative abundance of the diatom *Navicula decussis* was calculated in the absence of *Oscillatoria* spp., the gut content was approximately equal to that of the sediments. Although there were occasional instances in which the relative abundance of a particular taxon in the gut was significantly higher than in the environment, such an occurrence was probably based on nonrepresentative sampling of either the environment or lamprey population.

Digestion and Evacuation

Organisms were ingested by sea lampreys in proportion to their relative abundance in the tank. Mechanical damage to ingested organisms throughout the gut was not apparent. Although digestion of cellular contents appeared to occur as evidenced by the disappearance of chloroplasts, the test of all algae and virtually all microfauna remained intact.

Selective agglutination on the mucous band did not occur when sea lamprey larvae were offered the *Chlorophyta*, *Chlorella* sp., and *S. bijuga* and the diatoms *A. minutissima*, *A. ovalis* var. *pediculus*, *G. gracile*, *G. olivaceum*, and *S. ulna*. The relative abundance of each taxon was about the same anterior and posterior to the branchial openings. Selective agglutination of algae and the removal of other particles in bands of mucous through the branchial openings have been observed in the larvae of *L. planeri* and *E. danfordi* (Schroll 1959).

In the fecal material collected from 30 sea lamprey larvae (6–11 cm) removed from Shelter Valley Creek in July (17.8 C) and from 30 ammocoetes of similar size captured in January (0 C), 45 and 90%, respectively, of the algae examined had retained the colour of their chloroplasts. Within 48 hr of their removal from the feces, most algal taxa that had retained their chloroplasts survived and reproduced in Rodhe 8 medium (Rodhe 1948). Representatives of the diatom genus *Achnanthes* had the lowest survival rate in the gut (10–20%) while those of *Amphora* and *Cyclotella* had the highest (65–100%). Various species of Protozoa and Rotifera also passed through the gut without apparent harm. Presumably the digestive enzymes are less effective in their actions on some organisms than on others, particularly at low temperatures. Further, algae embedded in the mucous band may better resist the activities of digestive enzymes than those adhered to the surface. One must also not preclude the possibility that larvae gain at least some of their nutritional requirements from ingested bacteria, detritus, or even through the absorption of nutrients dissolved in the water. Concordant with these observations on lampreys, Le Fèvre (1942) found that a portion of the algae ingested by some cladocerans survived after evacuation.

Evacuation rates in the gut of larval sea lampreys did not differ among the taxa consumed. Complete evacuation required 54 hr (range 51–58 hr) at 16 C and 70 hr (range 60–75 hr) at 2.5 C regardless of larval length. Evacuation rate at the higher temperature was less than that for most teleosts, but at the lower temperature it was comparable (Beamish 1972; Tyler 1970).

Grazing

During both winter and summer, the number of algal cells found in the gut increased linearly with wet weight of sea lamprey larvae when expressed on a double logarithmic grid (Fig. 3). The regression

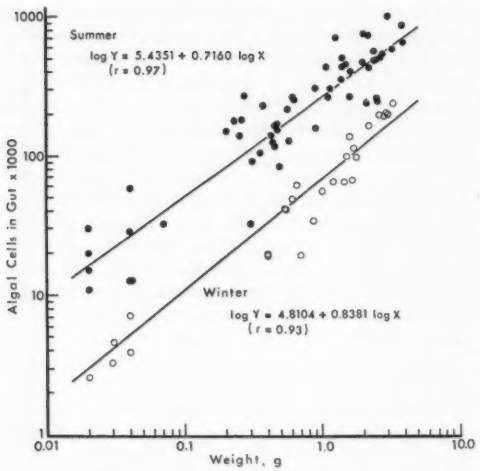

FIG. 3. Number of algal cells in the gut of sea lamprey larvae of different weight during summer (June) and winter (January).

coefficients, which were less than unity and not significantly different ($P<0.01$), using Bartlett's test for homogeneity (Steele and Torrie 1960), indicate that the number of algal cells consumed per unit weight decreased inversely with size. Thus, larvae of 0.02 g consumed almost thrice that ingested by a lamprey of 3.0 g when expressed on a unit weight basis. This may result from a relative reduction in surface area of the mucous band as larvae increase in size or a decrease in respiratory volume among the larger lampreys.

The position of the two regressions was significantly different ($P < 0.01$) when an analysis of covariance was applied (Steel and Torrie 1960). For a 3.0-g lamprey during the summer the number of ingested algal cells calculated from the regression was 5.8×10^5, considerably above the 1.7×10^5 estimated for a lamprey of similar size in the winter. The decrease in number of algal cells in the gut is undoubtedly related to a reduction in respiratory volume, which has been shown in *Ichthyomyzon hubbsi* to accompany a decrease in temperature (Hill and Potter 1970).

Number of algae ingested daily by larval sea lampreys at Shelter Valley Creek was calculated from the relation between gut contents and rate of evacuation. Larvae of 3.0 g ingested approximately $2.5–2.8 \times 10^5$ algal cells/day during summer, while ammocoetes of 0.02–0.03 g ingested about 0.7×10^4 cells/day. During the winter, number of algal cells ingested per day by 3.0-g larvae was $0.6–0.7 \times 10^5$ and by 0.02–0.03-g larvae, 0.2×10^4. Survival of evacuated algae would reduce the estimated net intake by about one-half in the summer and one-tenth in the winter. Approximately 1000 lampreys were taken by intensive electrofishing from a collection site at which previous collections had not been made and this is considered an estimate of the total number of lampreys at that site. Mean weight of the lampreys was 1.7 g. Hence, approximately 9×10^4 algal cells would be consumed and digested per day during summer by 1.7-g lampreys, giving 9×10^7 for the entire population. During the winter the figures would decline to about 5×10^3 and 5×10^6, respectively. The number of algal cells on the sediment of Shelter Valley Creek averaged $1.2 \times 10^6/cm^2$ in summer and $3.3 \times 10^5/cm^2$ in winter. The mean area of a lamprey collection site was 17.5 m^2. Hence, approximately 2.1×10^{11} algal cells were present on the sediment of a site during summer, and about 5.7×10^{10} during winter. Considering the amount of algae present in Shelter Valley Creek and the rate of feeding of lamprey, the effect of grazing by sea lamprey ammocoetes on the numbers of algae is negligible. Although detailed work on American brook lamprey is not available, the few samples taken suggest rate of evacuation and number of ingested algal cells to be about the same as that for sea lamprey; hence, feeding by American brook lamprey should have a similarly negligible effect on the algae.

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Feeding, Growth, and Productivity of Northwest Atlantic Harp Seals (*Pagophilus groenlandicus*)

D. E. SERGEANT

Fisheries Research Board of Canada
Arctic Biological Station, Ste. Anne de Bellevue, Que.

SERGEANT, D. E. 1973. Feeding, growth, and productivity of northwest Atlantic harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 30: 17–29.

The food of harp seals inhabiting the northwest Atlantic consists chiefly of pelagic fish, especially capelin, *Mallotus villosus*, and pelagic and benthic Crustacea (Euphausiacea, Mysidacea, Amphipoda, Decapoda), with smaller quantities of benthic fish. Feeding has been observed to take place on individual items by suction, and small fish are taken tail first. Feeding is intensive in winter and (by deduction) in summer, less intensive during spring and autumn migration, and in spring during whelping and moult. A weight loss in spring due chiefly to loss in thickness of subcutaneous fat (blubber) is most intensive in adult females as a result of lactation. This loss is made up slowly in summer. The preparturient females are partly segregated in midwinter on what may be the best feeding grounds. During lactation, and immediately following it when they are again segregated from other age-sex groups, adult females tend to feed on decapod Crustacea. In spring, the only time when all age classes are in the same geographic area, there is a stratification of feeding by size of organism and by depth, from chiefly Euphausiacea taken in surface waters by the weaned young, through capelin taken probably at intermediate depths by the immature animals, to herring, cod, and other groundfish taken by the moulting adults to depths of perhaps 150–200 m. Social feeding begins at about 1 year of age with the change from Crustacea to pelagic fish. From knowledge of the rate of feeding, body weights, and reproductive rates, the ecological efficiency of harp seals (i.e. weight of annual increment of population/weight of annual food eaten) is calculated at 0.005, a low figure. Annual weights of food items eaten by the northwest Atlantic population of harp seals are roughly estimated as: all organisms, 2×10^6 metric tons; capelin, 0.5×10^6 tons; and herring, 2×10^4 tons. Predation by harp seals on capelin stocks off eastern Canada occurs only during the winter months when pack ice is present as a resting substrate, the same resource being consumed in the summer months by the great whales (Balaenopteridae).

SERGEANT, D. E. 1973. Feeding, growth, and productivity of northwest Atlantic harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 30: 17–29.

La nourriture du phoque du Groenland dans le nord-ouest de l'Atlantique est constituée en majeure partie de poissons pélagiques, surtout le capelan, *Mallotus villosus*, de crustacés pélagiques et benthiques (Euphausiacea, Mysidacea, Amphipoda, Decapoda) et, en quantités moindres, de poissons benthiques. On a vu des phoques saisir des proies individuelles par succion. Les petits poissons sont attrappés la queue la première. Les phoques se nourrissent activement en hiver et aussi (par déduction) en été, à un degré moindre durant les migrations de printemps et d'automne, et durant la mise bas et la mue au printemps. La perte de poids au printemps, causée surtout par l'amincissement de la couche de graisse sous-cutanée, est la plus prononcée chez la femelle adulte, par suite de la lactation. La récupération a lieu lentement au cours de l'été. Il y a ségrégation partielle des femelles gravides avant la parturition à la fin de l'hiver sur ce qui peut être les meilleures aires d'alimentation. Durant la lactation et immédiatement après, alors qu'elles sont de nouveau séparées des mâles et des autres groupes d'âge, les femelles adultes se nourrissent surtout de crustacés décapodes. Au printemps, la seule saison à laquelle toutes les classes d'âge se trouvent dans une même aire, il y a stratification alimentaire selon la taille des proies et la profondeur: dominance d'euphausides capturés à la surface par les jeunes sevrés, capelan probablement mangé par les immatures à des profondeurs intermédiaires, et hareng, morue et autres poissons de fond capturés par les adultes en voie de mue à des profondeurs allant possiblement jusqu'à 150–200 m. L'alimentation sociale commence à l'âge d'un an environ, alors que les poissons pélagiques remplacent les crustacés dans le régime alimentaire. Connaissant le taux d'alimentation, le poids du corps et le taux de reproduction, nous pouvons calculer l'efficacité écologique de l'espèce (poids

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de la croissance annuelle de la population/poids de la nourriture consommée annuellement) et en arriver à une valeur de 0.005, qui est très basse. Le poids des proies consommées annuellement par la population de phoque du Groenland du nord-ouest de l'Atlantique est estimé comme suit: totalité des organismes 2×10^6 tonnes métriques, capelan 0.5×10^6 tonnes, hareng 2×10^4 tonnes. La prédation du phoque du Groenland sur les stocks de capelan de l'est du Canada ne se produit que durant les mois d'hiver, quand il y a des champs de glace solide où le phoque peut se reposer. Les grandes baleines (Balaenoptera) se nourrissent à même cette ressource en été.

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THE population of harp seals inhabiting the western North Atlantic is well-defined morphometrically (Yablokov and Sergeant 1963), while tag recoveries (Sergeant 1965) also show that it is relatively isolated from other populations occurring to the eastward.

The range of the western population extends from the Gulf of St. Lawrence in the south to Smith Sound (77°N) in the north, and from Hudson Bay in the west to Kap Farvel, Greenland, in the east. The population is highly migratory. In summer (June–November) it inhabits mainly the coasts of Davis Strait and Baffin Bay, in winter (December–May) coasts and bordering ice fields of eastern Canada south of Cape Chidley, Labrador (Sergeant 1965). The young are born in early March in the wintering range and are independent about 3 weeks later. The adults and immatures shed their hair coat (moult) in the same region during April.

The foods eaten by harp seals of this population in their winter range have been described briefly by Fisher and Mackenzie (1955), Myers (1959), and, among other topics, by Khuzin (1963). Some attention was paid to the summer foods of these harp seals in west Greenland by Nansen (1925), Dunbar (1949), Vibe (1950), Hansen and Hermann (1953), and Freuchen and Salomonsen (1958). I have added the observations of these authors to new data collected over the period 1952–71 in order to analyze feeding patterns through the year and during growth of the animals. Observations both in aquaria and in nature throw some light on feeding behavior. Analyses of growth and amount of food taken allow a first estimate to be made of the total food intake of the population. Intra- and interspecific feeding relations are then examined, including those presently occurring with man.

Material and Methods

Most harp seals taken in the water, whether by net, shooting, or hook and line, contain quantities of food remains.

By contrast animals taken on ice usually have empty stomachs. The latter have basked for some hours or days so that digestion is far advanced; only then, as a rule, are they dry and sleepy enough to be taken by stalking and shooting.

The most useful samples for food analysis are therefore of the first type. Our data of this kind are shown in Table 1, of the second kind in Table 2, both referring to seals taken in the wintering range. All available data from the summering range are shown in Table 3. Where two items were found in one stomach each was assigned a frequency of 0.5. No further quantification by volume was attempted.

For experiments in feeding, juvenile harp seals were captured at the icefields near the Magdalen Islands in midMarch 1971, at 2–3 weeks of age, and held in an enclosure, followed at ice-melt by an outdoor pool where they were fed herring and held till midMay. Feeding experiments were performed with isolated animals in plastic tanks at a disused oyster hatchery at Ellerslie, Prince Edward Island. Live, spawning rainbow smelts (*Osmerus mordax*) were captured in neighboring streams and live mysids *Mysis stenolepis* and shrimps *Crago septemspinosus* in the neighboring estuarine waters of Malpeque Bay.

Results

FOODS IN NATURE

Southward migrant seals in Labrador and the northern Gulf of St. Lawrence were sampled from netted catches. The animals were found to be feeding, sometimes lightly and sometimes heavily, on capelin (*Mallotus villosus*), euphausiid Crustacea, and herring.

Wintering animals in the Gulf of St. Lawrence were sampled over two seasons (January–April) near the confluence of the Saguenay River. These samples contained animals of all ages. The animals were shot in the water. Stomachs were nearly always well-filled, chiefly with capelin. Other pelagic fish, decapod and euphausiid Crutacea, and cephalopod Mollusca were occasional. The crustacean species, e.g. *Meganyctiphanes norvegica* and *Pasiphaea tarda*, suggested pelagic feeding.

Another winter sample (also January–April) was collected from the region of St. Anthony, Newfoundland. The animals were netted at coastal sites. Here the food was more variable, and decapod

TABLE 1. Frequency of different foods of harp seals (*Pagophilus groenlandicus*) on the Canadian east coast (seals taken in the water). Some earlier data from Fisher and Mackenzie (1955).

	Fish										Invertebrates									
	Capelin		Herring		Cod		Flatfish		Other		Decapods		Euphaus		Cephalop		Empty		Total	
Locality and date	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
										Southward migration										
Nain, Labrador																				
Dec. 13, 1965	1																2		3	
La Tabatière 1950																	100		100	
1951																	100		100	
1952			2														38		40	
1965	6												6				–		12	
	7		2										6				240		255	
										Wintering										
Saguenay–St. Lawrence																				
Jan.–Apr. 1969	88	90.7							1.5[a]	1.5	0.5	0.5	1.5	1.5	0.5	0.5[b]	5	5.3	97	100.0
1971	81	85.2							1.0[c]	1.1	3.5	3.7	3.0	3.2	0.5	0.5[d]	6	6.3	95	100.0
St. Anthony, Nfld.																				
1971	6.5	5.2	1	0.8	1	0.8	1.5	1.2	4[e]	3.2	55	43.6					57	45.2	126	100.0
Twillingate, Nfld.																				
1952	1																		1	
										Spring										
Magdalen Islands[f]			102	54.3	1	0.6	12	6.4	3	1.5[g]			2	1.1			68	36.1	188	100.0
Mar.–May 1956																				
										Northward migration										
Blanc Sablon																				
juv., June 1957	8																		8	
ad. & imm., Apr. 1953	1		12														91		104	

[a]Barracudina *Paralepis rissoi Kroyeri.*
[b]Octopus *Bathypolypus arcticus* (Prosch).
[c]Skate *Raja* sp. indet.
[d]Squid.
[e]Indet.
[f]An independent analysis of data on the same sample reported by Myers (1959).
[g]Redfish *Sebastes marinus.*

TABLE 2. Frequency of different foods of harp seals on the Canadian east coast (seals taken from ice fields). Much of the earlier data is cited from Fisher and Mackenzie (1955).

	Fish										Invertebrates							
	Capelin		Herring		Cod		Flatfish[a]		Other		Decap		Euphaus		Empty		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Juveniles																		
Magdalen Is. Apr.–May 1952											1						1	
N. Gulf, Apr. 6, 1966															6		6	
Front, Apr. 19, 1952	1												1		48		50	
Front Apr. 5–25, 1953													5[b]				5	
Immatures																		
Front Mar. 31, 1953	1																1	
Labrador Mar. 20–30, 1961	1																1	
N. Gulf Apr. 8, 1966															80		80	
Notre Dame Bay, Mar. 23, 1966	1																1	
Adult Males																		
60 m. E. Scatari Apr. 4, 1952					1										99		100	
Labrador Mar. 20–30, 1961 (Khuzin 1963)	1																1	
Front Apr. 6–16, 1954					2		1		1[c]								4	
Adult Females																		
S. Gulf Mar. 8, 1952											2				3		5	
S. Gulf Mar. 6–27, 1954	1		1								2.5[d]		0.5[b]				5	
Front Mar. 14–19, 1953											1		2		2		5	
Front Mar. 1961											4[e]						4	
Notre Dame Bay Mar. 30, 1966	1																1	

[a] *Hippoglossoides platessoides* and *Reinhardtius hippoglossoides*.
[b] *Thysanoessa raschi*.
[c] *Sebastes marinus*.
[d] *Pandalus montagui*.
[e] *Pandalus borealis*.

TABLE 3. Frequency of different foods of harp seals in the arctic.

Locality	Fish		Crustacea			Other	Total No.
	Capelin	Polar Cod[a]	Mysids	Euphausiids	Amphipods		
Frobisher Bay (Dunbar 1949)			1[b]				1
Cumberland Sd. 1967		2.5	1		0.5[c]		4
1969			1.5	2.5[d]	3.0[c]		7
Sleeper Is. Sept. 16, 1958			1[e]				1
Devon Is. Sept. 6, 1965		3					3
Canadian Arctic		5.5	4.5	2.5	3.5		16
Disco Bugt. (Nansen 1925)	++						
Thule (Vibe 1950)		1					1
General (Freuchen & Salomonsen 1958)	+++	+				+	
Godthaab (Dunbar 1959)	+++			++			
West Greenland	+++	+		++		+	

[a] *Boreogadus saida.*
[b] *Mysis oculata, M. mixta.*
[c] *Parathemisto libellula.*
[d] *Thysoanessa inermis, T. raschi.*
[e] *Mysis oculata.*

Crustacea were more frequent than fish. Identifications of the species in a series of 17 stomachs containing well-preserved material were as follows:

	% frequency
Pandalus sp., mostly *P. montagui*	81.5
Eualus fabricii	7.6
Argis dentata	4.2
Spirontocaris spinus	3.4
S. phippsi	1.7
Sclerocrangon boreas	0.8
Lebbeus polaris	0.4
Pasiphaea sp.	0.4
	100.0

These species indicate benthic feeding.

A third, quantitatively useful, sample came from adults at the Magdalen Islands in the southern Gulf of St. Lawrence in spring (March–May). Foods here were mainly fish, among which herring was the most common, and various flatfish (Pleuronectidae).

Last in the samples of Table 1, juvenile seals taken on the northward migration from the Gulf at two or three months of age contained capelin; older seals had mostly empty stomachs with some capelin and more herring.

The less complete data from seals taken on the ice (Table 2) add to coverage in location, time, and age-group. The young seals begin to take their first food in early April. This usually consists of Euphausiidae, particularly of the genus *Thysanoessa*, probably taken close to the floating pack ice, underneath which I have observed and collected specimens at this date. Some capelin is also taken, but this seems to be a much more typical food of the immatures. Lactating adult females at the "whelping patches" eat little but take some decapod Crustacea (especially *Pandalus borealis* and *P. montagui*). Moulting adults on ice east of Newfoundland have been noted as taking Atlantic cod (*Gadus morhua*) and flatfish in addition to capelin. It is also not rare to find the remains of larger, spinier species. In the Gulf of St. Lawrence I have seen the rejected heads of a sea raven (*Hemitripterusa mericanus*) and a shorthorn sculpin (*Myoxocephalus scorpius*) on the ice, the rest having been eaten, while on the ice east of Newfoundland intact redfish (*Sebastes marinus*) are often found on the ice near moulting harp seals.

The massed herds hauling out on the ice fields, especially on sunny days, in April and May seem to forage intermittently. In the northern Gulf of St. Lawrence in April 1966, herds of moulting adults and immatures left the ice fields for several days at a time, presumably to feed. East of Belle Isle in early May 1968, animals sampled from among a herd of adults and immatures had food remains in the hindgut only, indicating recent feeding apparently en masse.

Animals arriving at West Greenland in May–June (Sergeant 1965) feed heavily on capelin, with some euphausiids (Table 3). Hansen and Hermann (1953) write, and I translate:

"At the very time when the capelin concentrate in great shoals at spawning time, the harp seals arrive on migration from their breeding grounds at Newfoundland. The seals ... chase the capelin shoals greedily along the coast and the fjords. It is chiefly capelin which give harp seals their blubber layer in the course of the summer. One can also find capelin in the stomachs of seals during the winter."

The same foods are eaten by the young and immature animals which summer round Disco Island.

It is chiefly adult harp seals which summer in the colder waters of northwest Greenland and the Canadian arctic archipelago. These take arctic cod (*Boreogadus saida*) and various Crustacea including euphausiids, mysids, and the pelagic emphipod *Parathemisto libellula*. All food samples hitherto collected in the arctic from this population are, however, rather small.

Seasonal Changes in Feeding and in Fatness

Seasonal changes in feeding can best be deduced from data on seasonal fatness.

Seasonal fatness is measured either from blubber thickness or condition factor (girth × 100/length), as seen in Fig. 1 and 2. Both measurements show a rapid decrease in adult females during lactation and in both other age classes (immatures and adult males) during the moult, followed by a slow increase through the summer.

Some direct observations are available to support this picture of seasonal changes. Thus, at the Magdalen Islands the percentage of empty stomachs was seen to decrease steadily in both sexes, from March (the season of whelping) to May, indicating an increase in feeding intensity (Table 4). After this date the ice melts and the animals leave the area. The percentage of full stomachs in April and May is surprising in view of the rare examples of animals moulting on ice being found to contain food remains. One reason for the discrepancy is the difference in the nature of the samples discussed above. The real feeding rate must be intermediate between that derived from either sample.

Feeding Behavior

How prey is taken — Juvenile harp seals, fed smelt, shrimps, and mysids, took individual prey, largely by suction, which was audible if the seal's snout surfaced. The fish were swallowed tail-first, which accords with a commonly found position of capelin at the cardiac end of harp seal stomachs examined from the Saguenay area. The teeth were used to grasp only lightly. Nevertheless, large fish (e.g. *H. americanus*) must be bitten or ripped by the teeth.

Where prey is taken — Depth of feeding must at present be deduced from the food organisms ingest-

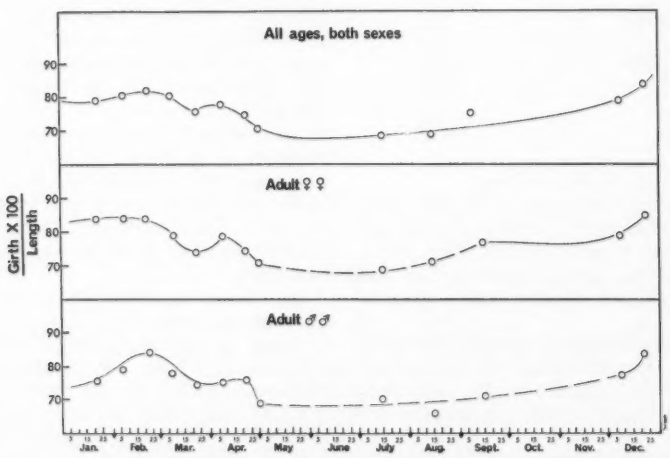

Fig. 1. Condition factor of harp seals through the year. Plotted points represent up to 50 animals.

ed. Templeman (1959, p. 73 and Fig. 43) considered that over the northeast Newfoundland shelf *S. marinus* are found deeper than 120 and, generally, 140 fath (220 and 256 m). I found fresh American plaice (*Hippoglossoides platessoides*) in stomachs of 20 adult harp seals on April 11, 1954, at 49°26′N, 50°05′W over a depth of 150–160 fath (274–293 m), 80–100 km from the nearest shallow water at Baccalieu Bank. These data suggest diving abilities of adult harp seals to as much as 250 m. In the last

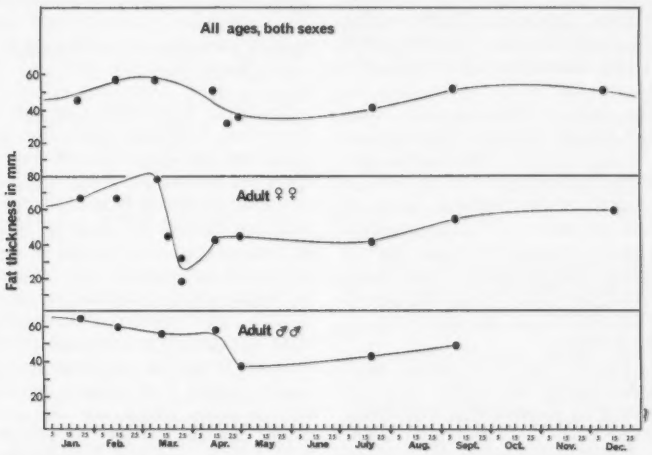

FIG. 2. Fat thickness of harp seals through the year. Plotted points represent up to 50 animals.

TABLE 4. Seasonal changes in feeding intensity of harp seals in spring, as shown by stomach contents of harp seals from the Magdalen Islands collected from March to May, 1956. F = females, M = males.

Time period	% of stomachs containing: Herring	Flatfish	Redfish	Gadidae	Invertebrates	% empty	Total no.
Mar.							
F	19.3	3.4	–	–	2.3	75.0	44
M	28.6	–	–	–	–	71.4	14
Apr.							
F	47.7	2.3	2.3	2.2	–	45.5	11
1–15							
M	57.1	28.6	–	–	–	14.3	7
Apr.							
F	76.7	11.7	–	1.6	–	10.0	20
16–30							
M	66.9	5.6	–	–	1.7	25.8	62
May							
F	92.9	–	7.1	–	–	–	7
M	73.9	10.9	8.7	2.2	–	4.3	23
Overall							
F	43.8	4.8	0.8	0.6	1.2	48.7	82
M	62.7	7.1	1.9	0.5	0.9	26.4	106
Both	54.3	6.4	1.5	0.6	1.1	36.1	188

example stomachs of immature seals were empty suggesting that they could not dive as deeply.

Social Behavior

Juvenile harp seals are solitary or indifferent to other animals through their first spring, summer, and winter, thereafter becoming highly social. This change in behavior correlates well with a shift in feeding from pelagic Crustacea to schooling pelagic fish.

In wintering seals, both in 1969 and 1971, a fine gradient in feeding behavior was seen from the sampling of hunters' returns (Fig. 3). The hunting area stretched 30 km from Escoumins in the east to Tadoussac and the mouth of the Saguenay River in the west. This part of the estuary of the St. Lawrence is known to be an area of intense upwelling (Hachey et al. 1957) and rich production and in winter tends to be clear of ice. Analysis of the 1969 catch showed young immatures concentrated in the eastern sector, adults in the western sector.

Both years' data showed that it was chiefly adult females which concentrated westwards (Fig. 3). It is therefore tempting to suppose that adult pregnant females seek out the richest feeding grounds, and that these are found in the Saguenay region at the western end of the feeding zone near Tadoussac. While detailed knowledge of productivity in this region is still lacking, the intensity of upwelling is greatest near Tadoussac where the shelf rapidly shallows.

Segregation of this kind was found by Wada (1969) in wintering northern fur seals (*Callorhinus ursinus*) off Japan. The adult females were found in deeper waters containing larger food organisms than where the juveniles were concentrated.

Food Consumption and Growth in Nature and in Captivity

Dr G. C. Ray, while Curator at the New York Aquarium, told the writer that, in his experience, adult seals eat about 5% of their body weight per diem and young seals about 10%. Detailed information for two young harp seals is available from the studies of Geraci (1971) at the Montreal Aquarium. In these studies two control animals were kept for 16 months on an ad libitum diet of smelt and herring supplemented with thiamin. Mean daily rations and weights were recorded each month (Table 5). Seasonal changes in feeding were marked, with maxima in July–November or December, and minima in March, which may indicate retention of a natural feeding cycle. There was no consistent cycle of weight change, however. The daily ration averaged 3.90–3.96 kg or 6.9–8.4% of body weight (mean =

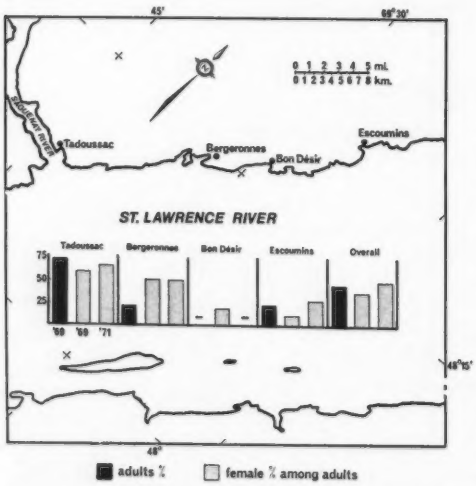

FIG. 3. Differential winter distribution of harp seals in the St. Lawrence estuary. For each locality of capture, dark hatching represents adult percentage, light hatching — percentage of females among adults.

TABLE 5. Weight gain and food intake of harp seals in captivity (after Geraci 1971).

		Seal no.			
		478		1867	
Month	Age (*months*)	S.wt.[a]	%C[b]	S.wt.	%C
Apr.	1	27	5.9	35	5.7
May	2	27	8.5	36	5.8
June	3	30	7.9	37	6.5
July	4	30	11.7	37	10.8
Aug.	5	32	11.3	41	9.8
Sept.	6	35	9.1	44	7.5
Oct.	7	37	10.5	48	8.8
Nov.	8	50	12.8	61	11.6
Dec.	9	63	10.0	71	5.8
Jan.	10	68	6.3	78	5.4
Feb.	11	72	5.3	79	4.2
Mar.	12	77	3.2	80	3.5
Apr.	13	70	5.1	81	4.4
May	14	68	6.9	81	6.4
June	15	65	11.7	83	7.6
Mean			8.4		6.9

[a]S.wt. = seal weight in kg.
[b]%C = food intake: mean percent of body weight consumed per day.

7.6% for both animals). These results for two immature animals support the general conclusions of Ray.

Highest weights of stomach contents recorded in nature were 1.75 kg for a one-year-old seal of 40-kg estimated body weight; and 4.7 kg for an estimated 100-kg adult — or about 4.7% of body weight in each case. The frequency and duration of feeding episodes in the wild are unknown. In the estuary of the St. Lawrence digested capelin was usually found in the pylorus with fresh capelin filling the rest of the stomach. This circumstance suggested extended periods of feeding.

The captive animals showed higher growth rates than in nature, reaching 65–83 (mean, 74) kg at 1½ years instead of at 3–4 years as in nature (Fig. 4), and appeared obese. Indeed, in one of the two animals, dead after 2¾ years, the condition factor was at the high level of 107. However, its length at 142 cm was also high so that some of the increased growth may have been in carcass weight as well as in fatness. The captive seals, as well as being supplied with abundant food, were confined, leading to less exercise, and were maintained at a higher ambient temperature than in nature. Nevertheless, the suspicion arises that feeding in nature may often be less than optimal. Indirect evidence bearing on this subject is discussed below (p. 26).

Production and Ecological Efficiency

Age determination of harp seals from growth layers in the canine teeth is accurate and allows study of age-specific growth rates (Fig. 4).

Since a good deal of the harp seal's weight is blubber which thickens rapidly in early development and later varies seasonally, the estimate of biomass is rather arbitrary. Production could be estimated starting from the weight of newborn young (about 9 kg), but I shall use as a starting point the mean weight of the weaned young (about 35 kg). Mean weight of adults and immatures in spring is about 100 kg. In harp seals, adults represent about 50% of the total population of adults and immatures just before birth of the year's crop of young. The sexes are equal in numbers, and about 90% of the adult females give birth annually. Annual production is therefore equal to $0.25 \times 0.9 = 0.225$ of the population and annual production (of weaned young) in weight is $0.35 \times 0.225 = 0.07875$ of unit biomass.

Annual food consumption by an immature or adult harp seal is assumed to be 5 kg daily. Allowing for fasting periods in spring this might be maintained for 300 days in the year, giving an annual food consumption of about 1500 kg (1.5 metric tons), or 15 kg for every kg of seal.

Ecological efficiency is defined as yield or organism divided by its food intake (Paloheimo and Dickie 1970). This, for harp seals, is therefore estimated at $0.07875 \times 1/15 = 0.00525$, or about 0.5%. A common value of ecological efficiency at lower trophic levels is about 10%. Harp seals (as perhaps other sea mammals) are therefore inefficient converters of fish flesh. Elephants (*Loxodonta africanus*) have a food conversion efficiency (measured as growth/food consumed i.e. equivalent to the para-

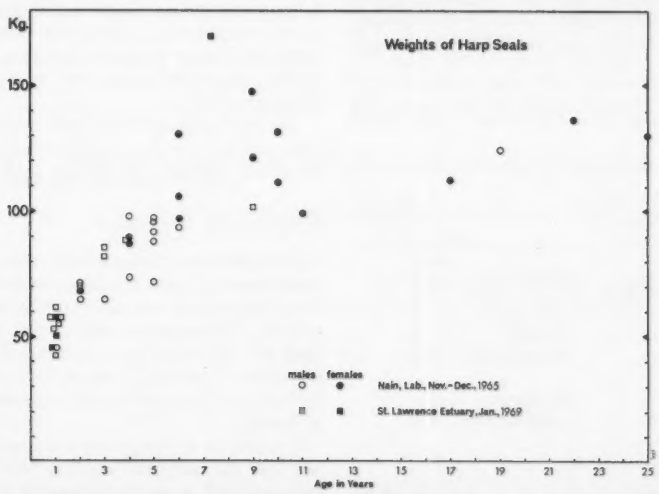

FIG. 4. Age-specific growth in weight of free-living harp seals.

meter measured above) of 0.005, deer (*Odocoileus virginianus*) of 0.012, and cattle (*Bos taurus*) of 0.060 (Petrides et al. 1968).

Discussion

Food Consumption and Intraspecific Relations

How much food is consumed by harp seals? The population in the northwest Atlantic in the late 1960's produced about 300,000 young annually (Sergeant 1971b), necessitating a stock size of about $1\frac{1}{3}$ million animals just before birth of the young. At an annual feeding rate of 1.5 tons per animal, this population would consume about 2 million tons of food per annum.

A very crude estimate of the overall composition of the food is shown in Table 6. This is obtained by summing the number of times each food item was found, each sample yielding one occurrence of any item found in it. According to this analysis, fish constitute some 60% of the food, and capelin alone some 25%.

On this basis, the northwest Atlantic harp seals eat some half million tons of capelin annually. Judging by respective residence times of the seals, they eat most of this (perhaps 0.4 million tons) during the winter in the eastern Canadian region, the remainder in west Greenland waters. This resource is shared with many other predators, such as the balaenopterid whales, as discussed below. For comparison, about 1.5 million tons of capelin were caught by Norway in the northeast Atlantic (Barents Sea, etc.) in 1970, and about 0.2 million tons by Iceland, (O. Dragesund personal communication). The northwest Atlantic fishery has until very recently been negligible — 3574 tons in 1968, for example (Anon 1970).

Table 6. Frequencies of different food items found in harp seals.

Fish	No. of occasions		No. of occasions
		Crustacea	
Capelin	16 (26%)	Euphausiids	9
Herring	5	Decapods	7
Cod	4	Mysids	4
Polar cod	4	Amphipods	2
Flatfish	3		
Redfish	2	*Mollusca*	
Skate	1	Cephalopods	2
Barracudina	1		
Indet.	1		
	37 (61%)		24 (39%)

These estimates of food consumption suggest that availability of food could be a limiting factor for harp seals. Indirect evidence comes from studies of age at maturity (Sergeant 1966). As the exploited harp seal populations have decreased in recent decades, age at first maturity has decreased. Since age at first maturity is a function of growth (Laws 1959), growth has improved over this period. There is a strong suspicion that improved growth has resulted from improved nutrition. While I have not been able to demonstrate either improved growth or improved nutrition over the years, I have observed greatly improved condition of moulting animals between the early 1950s and late 1960s with disappearance of cracked skin in adult males at the height of moult in late April. Probably blubber thickness has increased over the same period but the data are not sufficient for critical testing.

If food was, or is, limiting to the population one would look for mechanisms reducing intraspecific competition. These seem to exist in the form of feeding segregation in either the vertical plane (adults diving more deeply) or the horizontal plane (geographical segregation on a greater or lesser scale). In spring all age classes are concentrated in a reduced range and here segregation by depth appears to be most marked with adults alone eating the larger food organisms (herring in the Gulf, groundfish off eastern Newfoundland), with some segregation by area (adults remaining among the ice of the whelping ground, immatures at its southern fringe). On arrival in west Greenland there is a temporary concentration of all age-sex classes on the abundant spawning capelin, but by high summer the adults have dispersed into high arctic waters leaving the young immatures in subarctic waters. This large-scale spatial separation persists into winter when the young immatures delay their migration southward (Sergeant 1965).

The increasing sociality that occurs after the first year may in some way result in more effective feeding on active, pelagic fish species such as herring.

Interspecific Relations

Since the pelagic food resources (decapod Crustacea, capelin, herring, and groundfish) in the eastern Canadian region are resident, why do the harp seals undergo long seasonal migrations? Why do they not feed on the abundant spawning capelin at Newfoundland in early summer as they do at Greenland? So long a migration necessitates heavy expenditures of energy.

Looked at in the present context, one can only say that the harp seal requires both a pelagic food resource and a resting substrate of pack ice, and can only persist where both are present, so that

migrations are forced on it to the degree that seasonal changes occur in ice distribution. As usual, exceptional situations are revealing. Very rarely, young harp seals attempt to summer in the Gulf of St. Lawrence or on the east coast of Newfoundland. Usually, these young animals are associated with Atlantic harbour seals (*Phoca vitulina concolor*). Only two animals are well-documented. A juvenile taken at Escoumins on the north shore of the Gulf of St. Lawrence on June 30, 1969, had plant material in the stomach (the species *Triglochin maritima*, *Glaux maritima*, and possibly a sedge family Cyperaceae) indicating that it had fed around a sandy or salt-marsh coast. Its girth and fat thickness were abnormally low. A tagged juvenile killed at Bay of Islands, Newfoundland, in August 1966 had a carcass weight of 10 kg, about half the normal weight. One cannot distinguish from these data whether abnormally small animals failed to migrate, or whether animals that failed to migrate suffered unusually poor growth, but the latter seems more likely.

This evidence suggests that young harp seals cannot find suitable feeding conditions close to coastal resting sites. The rarity of the episodes demonstrates the accuracy of the north-seeking migratory behavior of young harp seals in spring (Sergeant 1971a), which in turn indicates intense selection over a long period towards the migratory habit.

Viewed over the evolutionary time-span, one may suppose the harp seal with its white-coated pup to have had a long association with ice. It moves southwards into open water only in early winter in search of the rich, boreal stocks of pelagic fish and euphausiids, suggesting a barrier to further southward dispersal.

Species such as fin whale *Balaenoptera physalus* (Jonsgaard 1966) and minke whale *B. acutorostrata* (Sergeant 1963) are major consumers of capelin and euphausiids in the northwest Atlantic. The cold-adapted minke whale migrates north in spring, close behind the migration of the harp seals, and south in the fall, shortly ahead of the seals. Harp seals enter the Newfoundland–Gulf of St. Lawrence area in December and depart in April or May, the great whales departing in November and arriving in May or June.

Several documented episodes of stranding of fin whales by ice on lee shores (Sergeant, et al. 1970) show that selection works against whales that linger in waters liable to be covered with pack ice. In west Greenland, at the period when the harp seals feed on capelin, there is a notable lack of fin whale catches although sperm whales (*Physeter catodon*) were caught in May–June.

Two hypotheses are therefore available to explain the reciprocal distribution of seals and whales feeding on the same capelin resource: (1) each exploits the niche when it can — the seals when there is ice, the whales when there is none — and no competition occurs. Food is not limiting. (2) There is limited competition between them, and collectively they are food-limited, which limits total numbers of each.

If there is drift ice in southwest Greenland in May–June when the seals arrive, having left the Newfoundland capelin stocks, (1) is probably true; if there is no drift ice, (2) is probably true. A study of the Danish Ice Atlas (Det Danske Meteorologiske Institut 1928–) shows that ice is typical in this region, suggesting that (1) is true. Probably the seals haul out on the ice to rest after the long journey across Davis Strait.

A further test will come from intensive human exploitation of capelin expected in the next few years. If food is limiting to the natural predators, they will suffer further food shortage when man is added as a predator and the populations that can be maintained will be lower than hitherto.

TABLE 7. Calculated consumption of herring in the Gulf of St. Lawrence by harp seals, with feeding rates from Table 4.

Month	Supposed population present	Proportion feeding	Individual daily consumption (*kg*)	Days	Tons consumed
Mar.					
2nd half	250,000	0.25	5	15	4687
Apr.					
1st half	200,000	0.50	5	15	7500
2nd half	100,000	0.75	5	15	5625
May					
1st half	50,000	1.00	5	15	3750
Total					21,562

Interaction with Man

Turning the last problem around, do harp seals interfere greatly with predation by man or by how much do harp seals reduce the sustainable yields of individual fishery resources to man?

The species of invertebrates eaten by harp seals in the northwest Atlantic are, for the most part, noncommercial. The diversity of decapod Crustacea taken does not indicate much interference with the new and growing human fishery for *Pandalus borealis*. Capelin is not, as yet, fished in large tonnage in the northwest Atlantic. Consumption of herring by harp seals is much less than of capelin. It occurs mainly in the Gulf of St. Lawrence, where about one-third of the harp seal population, or some 450,000 animals, live. Herring is there taken mainly by the adults (about 250,000 animals). Also, during the season of herring feeding at the Magdalen Islands, the animals are gradually moving north as the ice disappears to be replaced as predators of herring by fin and other species of whales. Combining the increasing intensity of feeding in spring with arbitrary rates of emigration between March and May results in an estimated herring consumption by harp seals around the Magdalen Islands of about 20,000 tons (Table 7). This compares with a human fishery in the Gulf of St. Lawrence in 1968 of 100,000 tons (Anon. 1970), which may have overtaxed the stocks since subsequent catches have declined.

The estimate of herring consumption may be too high because, in many years, disappearance of the ice and northward migration of the seal take place a month earlier, by mid-April; in the sampling year (1956), the ice persisted into May near the Magdalen Islands. However, some harp seal herring feeding that cannot be quantified was recorded also in the northern Gulf and this could increase the estimate to a higher level.

Acknowledgments

Hr E. Vangstein, Sandefjord, supplied whaling statistics for West Greenland. In 1965 Dr M. M. R. Freeman sent details of harp seal feeding from Grise Fjord, Ellesmere Island, and Mr H. A. Williamson sent the same from Nain, Labrador. Collections and analyses of stomach contents were made at the Magdalen Islands in 1956 by Mr P. Montreuil who gave access to original data and to unpublished information on feeding of captive harp seals at the Montreal Aquarium in 1971. Dr T. G. Smith collected data in Cumberland Sound, Baffin Island, in 1968, and he and Mr W. Hoek initiated collections in the St. Lawrence estuary in January 1969, which were continued by purchase from hunters. Dr D. E. McAllister, National Museum of Natural Sciences, Ottawa, identified the fishes; Dr M. C. Mercer, Fisheries Research Board Biological Station, St. John's, Nfld., the Cephalopoda; Dr E. H. Grainger, Fisheries Research Board Arctic Biological Station, Ste. Anne de Bellevue, Que., the decapod Crustacea; and Dr D. E. Swales, Macdonald College, Que., the plant material. To all these people I am very grateful.

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Endrin Toxicosis in Rainbow Trout (*Salmo gairdneri*)

BLAKE F. GRANT AND PAUL M. MEHRLE

Fish-Pesticide Research Laboratory
Bureau of Sport Fisheries and Wildlife, United States Department of the Interior,
Columbia, Miss. 65201, USA

GRANT, B. F., AND P. M. MEHRLE. 1973. Endrin toxicosis in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 31–40.

In mature rainbow trout (*Salmo gairdneri*) receiving sublethal doses of endrin (4.3–145 μg/kg body wt/day in 0.215–7.25 mg/kg of food) for 163 days and then forced to swim for 1 hr, the insecticide affected serum electrolytes, osmolality, total protein, cholesterol, cortisol, lactate, glucose, liver glycogen, and growth. Forced swimming alone altered 9 of 16 serum parameters examined. Apparent increases in serum Na and Cl and significant increases in osmolality and liver glycogen were directly related to dosage. A biphasic distribution of phosphate, total protein, and cholesterol with dosage was apparent. Glucose was increased about 50% by 145 μg/kg but was unaffected by lower doses. Variance analysis of zone electrophoretic patterns disclosed an in teraction between serum protein distribution and dose. Mobilization of liver glycogen was apparently inhibited by low doses and almost totally blocked by high doses. Correspondingly, trout given 14.5 μg/kg or more had lowered serum cortisol levels whereas the lowest dose elevated cortisol. Growth was inhibited appreciably by 145 μg/kg but not by lower doses. Visceral fat accumulated 4.8–8.7 μg endrin/g tissue in the 43 and 145 μg/kg exposures. We conclude that endrin caused dysfunction of physiologic processes critical to survival.

GRANT, B. F., AND P. M. MEHRLE. 1973. Endrin toxicosis in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 31–40.

Des doses sublétales d'endrine (4.3–145 μg/kg de poids du corps par jour = 0.215–7.25 mg/kg de nourriture) administrées à des truites arc-en-ciel (*Salmo gairdneri*) affectent les électrolytes sériques, l'osmolalité, les protéines totales, le cholestérol, cortisol, lactate, glucose, glycogène hépatique et la croissance. Nous avons examiné ceux-ci et d'autres paramètres physiologiques après exposition de 163 jours et nage forcée d'une heure. L'augmentation significative du Na et du Cl sériques, de l'osmolalité et du glycogène hépatique est directement liée à la dose. La distribution biphasée du phosphate, des protéines totales et du cholestérol est, elle aussi, nettement associée à la dose. Le glucose augmente d'environ 50% à une concentration de 145 μg/kg, mais n'est pas affecté par des doses inférieures. L'analyse de la variance de la zonation électrophorétique révèle une interaction entre la distribution des protéines sériques et la dose. La mobilisation du glycogène hépatique durant la nage forcée est apparemment ralentie par de faibles doses, et presque totalement bloquée par de fortes doses. Egalement, les truites exposées à des doses de 14.5 μg/kg ou plus ont un bas niveau de cortisol sérique, alors qu'une dose plus faible élève la teneur en cortisol. Il y a diminution sensible de la croissance à 145 μg/kg, mais non à des doses plus faibles, et l'endrine ne cause pas de mortalités. La graisse viscérale emmagasine de 4.8 à 8.7 μg d'endrine/g de tissu à la suite d'expositions à 43 et 145 μg/kg. Nous en concluons que l'endrine cause une rupture des processus physiologiques, mettant en jeu la survie. La nage forcée, à elle seule, affecte 9 des 16 paramètres sériques examinés.

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ENDRIN[1] in water is reported to be extremely toxic to fishes (Ferguson et al. 1965; Saanin 1960; Tarzwell 1965). It is relatively long-lived; Nash and Woolson (1967) reported a half-life of approximately 11 years in soil. It is widespread in the United States; Henderson et al. (1969) reported that fish from half of the sampling stations in the U.S. had endrin residues in the range 0.01–1.5 ppm ($\bar{x}$ = 0.07 or approximately 0.28 mg of endrin/kg dry weight). Recent reviews (Johnson 1968; Katz et al. 1969) of pesticides as aquatic pollutants point out the

[1](1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene).

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need for studies of chronic exposures of fishes to environmental levels of pesticides.

Mount (1962) reported that endrin affected growth, reproduction, and the central nervous system in guppies (*Poecilia reticulata*) and bluntnose minnows (*Pimephales notatus*). The latter effect was indicated by altered respiration rate, abnormal behavior, hypersensitivity, and convulsions.

Eller (1971) observed that chronic exposures of cutthroat trout (*Salmo clarki*) to endrin by bath or in food caused hyperplasia of the islets of Langerhans. His observations implied that carbohydrate metabolism was altered by endrin. The histopathology was not confirmed by supporting evidence such as biochemical analysis, however. Eisler and Edmunds (1966) reported an atypical electrolyte distribution between blood and liver that suggested liver impairment in northern puffers (*Sphoeroides maculatus*) acutely exposed to endrin. Grant and Mehrle (1970) reported other altered physiologic parameters of growth, reproduction, thyroid activity, intermediary metabolism, and osmoregulation in goldfish (*Carassius auratus*) chronically exposed to endrin by diet (4.3–430 µg endrin/kg body wt/day). The studies cited indicate that endrin toxicosis is a complex syndrome and more information from studies involving sublethal exposures is desirable if we are to predict safe environmental levels.

In the present study, we were interested in determining dietary intakes of endrin resulting in disturbances in growth rate, osmoregulation, and intermediary metabolism. To detect disturbances more readily, we evaluated the ability of endrin-exposed fish to adjust to the moderate physiologic demands of a "loading" stress as may occur in nature. We chose forced swimming, which in healthy trout may not be stressful per se (Fagerlund 1967).

The dietary concentrations (0.215–7.25 ppm) were selected to be sublethal and they represented a slightly higher range of concentration than a piscivorous fish may encounter in nature if one compares the doses to Henderson's (Henderson et al. 1969) national monitoring data cited above (0.04–6.0 ppm, whole body dry weight). However, piscivorous species are exposed to an additional amount of endrin in water.

Materials and Methods

Experimental — Year-old rainbow trout (*S. gairdneri*) obtained from the National Fish Hatchery, Neosho, Missouri, were maintained, 10–12 each, in six 490-liter fiberglass tanks with 4–6-liter/min inflows of aerated well-water at 16–17 C. Light from skylights during November–April was supplemented with 8 hr/day of fluorescent light. The trout were fed a pelleted diet[2] at a rate of 2% body wt/day with feeding adjustments for weight gain approximately each month. Concentrations of 0, 0.215, 0.725, 2.15, and 7.25 mg of endrin (99% purity, Shell Chemical Co.)/kg of food were prepared by using corn oil for a carrier. These preparations were used to dose the six groups of 0 (duplicate control groups), 4.3, 14.5, 43, and 145 µg/kg body wt/day.

After 163 days, the endrin-fed trout and half of each of the two control groups were forced to swim, 5–6 fish at a time, for 1 hr in a stamina tunnel against a current of 1.5 ft/sec. Immediately after forced swimming, the fish were anesthetized in M.S. 222 (0.2 g/liter) and blood was drawn from the caudal artery. The *p*H was determined immediately on 20 µliter of whole blood with microelectrodes. The remaining blood was allowed to clot in centrifuge tubes at room temperature (20 C) for 45 min. No longer than 3–4 min elapsed from beginning anesthesia for all five or six fish until the *p*H was determined on the last individual of the test run. The retracted clot was centrifuged and the serum was decanted and centrifuged again. A portion of the serum was analyzed for glucose and the remainder was frozen for other, subsequent analyses. Body, gonad, and liver weights were recorded. Whole livers were taken for glycogen estimation and heated to 70 C to stop glycogenolysis immediately after the blood was drawn. Samples of mesenteric fat were collected for endrin analysis. Endrin concentrations were estimated by electron-capture gas–liquid chromatography (Tindle 1972).

Analytical — Ultramicromethods used for serum analysis gave excellent reproducibility and accuracy. The error of reproducibility in all methods on 10 replicates was less than 1% and the error of accuracy was less than 5%. Sodium and potassium were analyzed with an internal standard, flame photometer. Chloride was analyzed with a Cotlove Cl titrator. Calcium and magnesium were analyzed with an atomic absorption spectrophotometer. Free phosphate in the protein filtrate was estimated by the Fiske and Subbarow (1925) method with volumes scaled down to accommodate 20 µliter of serum. For the colorimetric or absorptiometric methods, we used a Beckman DU-2[3] spectrophotometer equipped with an RHO microcell adapter, a Beckman Ultramicro Analytical System, or a Klett–Summerson colorimeter. We estimated osmolalities with a Hewlett–Packard 302 vapor pressure osmometer. No difference in osmolality of selected samples estimated before and after freezing was detectable. Total protein was estimated on the serum filtrate (10% trichloroacetic acid) by a modification (Bailey 1967) of the Folin–Ciocalteau method. Sera were analyzed electrophoretically with the Beckman Microzone System using Beckman or Brinkman cellulose acetate membranes. The integrated areas of easily recognized zones (Fig. 1) in the densitometer tracing

[2]Cortland #7, personal communication, Dr A. M. Phillips, Cortland, N.Y.

[3]Mention of products by commercial or trade names does not constitute endorsement by the Fish and Wildlife Service or Department of the Interior.

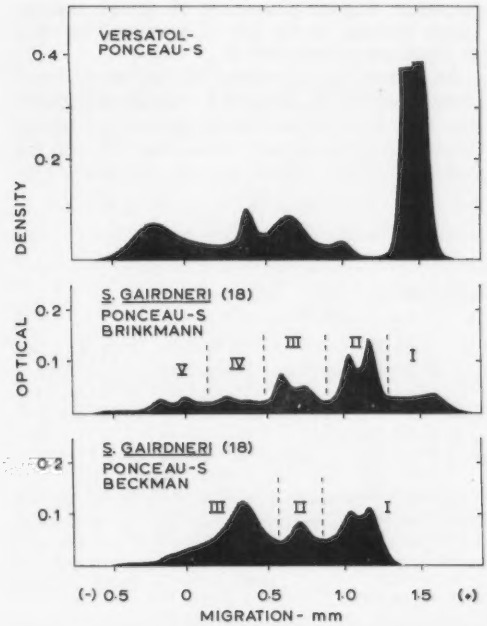

FIG. 1. Electrophoretic patterns showing zones integrated for comparison of relative amounts of protein. Parallel runs of serum from fish #18 show difference in resolution of two cellulose acetate membranes. Human control serum Versatol® was run on the Brinkman membrane. The conditions were the same on all runs: 250 volts, 25 min, veronal buffer, $\mu = 0.075$, $p\text{H} = 8.6$.

were compared within experimental groups. Total cholesterol from the serum filtrate was estimated by Zak's method (1957) as adapted for ultramicro amounts (Beckman Ultramicro Analytical System). Nonprotein nitrogen was estimated after Kjeldahl digestion by the "alkaline phenate" reaction with the modifications of Chaney and Marbach (1962). Cortisol was estimated fluorimetrically (Baird-Atomic Fluorispec, SF-1 equipped with microcells) by scaling down the volumes used by Mattingly (1962). Lactic acid was estimated by measuring the extinction (366 nm) of NADH simultaneously generated with the enzymatic oxidation of lactate (Biochemica Test Combination, Mannheim, Germany). Glucose in the serum filtrate was estimated enzymatically by the methods of Keston (1956) and Teller (1956), using Glucostat (Worthington Biochemical Corporation, Freehold, New Jersey) and following the manufacturer's recommended procedure. Liver glycogen was estimated by a modification of the method of Montgomery (1957). Liver samples were dissolved in hot 30% KOH. Glycogen was precipitated with 95% ethanol, centrifuged, and the precipitate was hydrolyzed in 2-N sulfuric acid at 100 C. Phenolsulfuric acid solution was used to develop color.

Mean comparisons using the least significant difference and variance analysis (Snedecor and Cochran 1967) were applied to the data. The Na, Cl, and osmolality values were also analyzed by linear regression. Fisher's t test was used for mean comparisons of values between exercised controls and nonexercised controls.

Results

Physiologic characteristics of exercised and nonexercised trout — Nine of the 16 serum constituents analyzed were significantly altered by forced swimming (Table 1). In addition, liver glycogen decreased. Potassium, Mg, amino acids, cholesterol, and proteins decreased, whereas osmolality, glucose, lactate, and cortisol increased. Most of those constituents having the greatest change, i.e. amino acids (−55.9%), glucose (+56.2%), and lactate (+47.4%), are directly involved in the energy transformations of exercise. Cortisol, which changed most (+696%), is involved in stimulating glycogenolysis, though not directly as an energy source.

Growth and visual observations — All groups of trout, controls and dosed fish, gained weight during endrin treatment (Table 2). Only the highest endrin dose, 145 µg/kg, reduced the growth rate, the gain after 163 days being about half that of the control group.

Endrin caused no mortality and the only symptom suggesting endrin toxicosis, seen consistently in the 145-µg/kg group and occasionally in the 43-µg/kg group, was hyperexcitability. All endrin-treated trout showed increased swimming activity. Occasionally, startled individuals in the 145-µg/kg group swam into the tank wall with sufficient force to cause injury. However, individuals of the latter group never showed the reported symptoms, convulsions and loss of equilibrium, associated with terminal endrin toxicosis (Mount 1962).

Major serum constituents and physicochemical characteristics of exercised trout receiving endrin (Fig. 2) — In exercised fish, direct but low degree of correlation ($r = 0.25$) existed between serum Na and the endrin dose ($0.05 > P > 0.025$). The Na concentration corresponding to the 145 µg/kg dose represented an increase of 3.5% in Na. Also, serum Cl correlated directly ($r = 0.24$) with endrin dose ($P < 0.05$). The Cl concentration corresponding to the 145-µg/kg dose represented an increase of 3.1% over that of the exercised controls. These variations in Na and Cl are, however, only 6.4% (0.25^2) and 5.8% (0.24^2) attributable to variation in dose. Serum osmolality of the endrin-treated trout, like Na and Cl, was directly related to the

dose ($P < 0.005$). The osmolality corresponding to the 145-μg/kg dose represented a 4.4% increase over that of the exercised controls. Variance analysis, a more stringent test for correlation of Na and Cl concentration with dose, indicated nonsignificance. Variance ratio between osmolality and dose, however, was significant. Sodium with balancing anions accounted for essentially all of the serum osmotic pressure in all groups and apparent increases in Na and Cl accounted for 70% of the increased osmolality.

An inverse relation existed between serum phosphate levels and the doses of 4.3 μg/kg and greater. The 4.3-, 14.5-, and 43-μg/kg groups had significantly higher phosphate levels than that of the exercised controls. Serum Mg was also affected by

TABLE 1. Physiologic characteristics of exercised and nonexercised rainbow trout.

	Nonexercised ($n = 10$)	Exercised ($n = 11$)
Na (*mEq/liter*)	163.5±1.2[a]	163.7±2.6
K (*mEq/liter*)	1.88±0.18	1.34±0.14[b]
Cl (*mEq/liter*)	121.8±1.7	124.1±1.8
Osmolality (*milliosmols/liter*)	302.4±1.5	308.4±1.8[b]
Ca (*mEq/liter*)	6.53±0.34	6.42±0.23
PO_4 (inorganic, *p*H 7.2) (*mEq/liter*)	2.94±0.14	2.48±0.22
Mg (*mEq/liter*)	2.71±0.15	2.10±0.08[b]
*p*H	7.264±0.015	7.225±0.008
Nonprotein nitrogen (*mg/100mliter*)	22.5±0.6	21.9±1.4
Creatinine (*mmole/liter*)	0.136±0.005	0.141±0.003
Total amino acid[c] (*mmole/liter*)	10.9±1.4	4.8±1.1[b]
Cholesterol (*mmole/liter*)	14.1±0.9	10.8±0.7[b]
Glucose (*mmole/liter*)	3.33±0.40	5.22±0.17[b]
Lactic acid (*mmole/liter*)	13.5±1.4	19.9±2.0[b]
Cortisol (*mmole/liter*)[d]	0.24±0.12	1.91±0.68[b]
Liver glycogen (*mg/g dry liver*)	182±36	16.5±3.4[b]
Total protein (*g/100 ml*)	4.03±0.19	3.41±0.21[b]
HSI (%)[e]	1.07±0.06	1.01±0.03

[a]Mean and standard error.
[b]Significantly different from nonexercised controls (Fisher's *t* test, $P < 0.05$).
[c]Total α-amino nitrogen, leucine equivalent.
[d]$n = 5$, sera pooled, two fish each.
[e]Hepatosomatic index = liver weight/body weight (100).

TABLE 2. Gains in weight (*g*) of rainbow trout during 163 days of exposure to endrin at various rates.

	Dose (μg/kg body wt/day)				
Time	Control (n=21)	4.3 (n=12)	14.5 (n=12)	43 (n=10)	145 (n=10)
Weight at start[a]	199±89	165±55	180±51	177±48	168±34
Gain at 124 days	193±45	174±24	177±27	170±22	53±14[b]
Gain at 163 days	274±111[c]	265±68	256±70	259±63	109±40[b]

[a]The standard deviation is given for the beginning weights to illustrate this "constant" or population parameter; the standard error is given subsequently to illustrate the accuracy of the mean.
[b]Significantly different from controls ($P < 0.05$, Fisher's *t* test).
[c]Eleven of these trout were exercised before their weights were taken. The difference in weights was nonsignificant and the values were pooled.

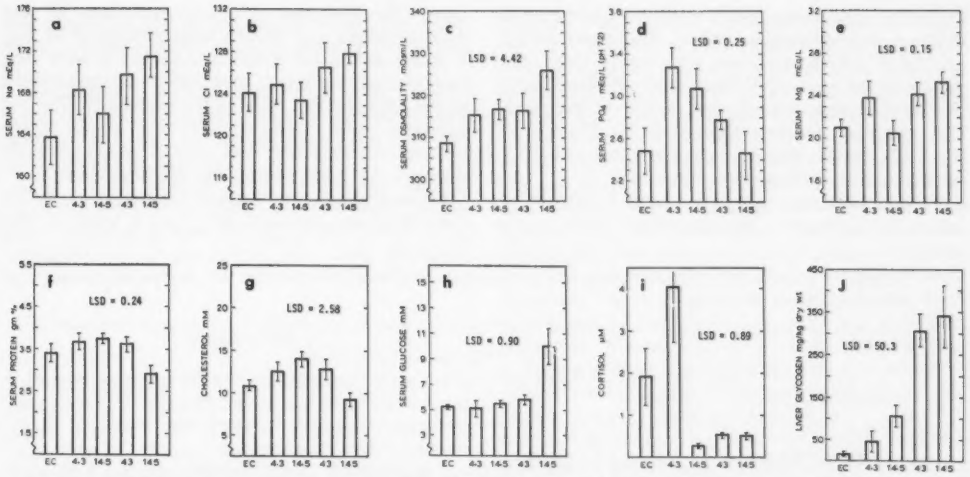

FIG. 2. Major physiologic characteristics (means and standard errors) of exercised (1 hr) trout fed endrin (0–145 µg/kg body wt daily) for 163 days. EC, exercised control. LSD, least significant ($P < 0.05$) difference. Serum Na: $Y = 166.2 + 0.0405\,X$, $r = 0.254 \pm 0.130$, $n = 52$, $0.05 > P > 0.025$, X = dose. Serum Cl: $Y = 124.2 + 0.027\,X$, $r = 0.240 \pm 0.129$, $n = 53$, $0.05 > P > 0.025$. Serum osmolality: $Y = 312.7 \pm 0.0965\,X$, $r = 0.417 \pm 0.114$, $n = 53$, $0.005 > P > 0.0005$.

endrin. Although the value for the 14.5-µg/kg group lay outside the trend, variance analysis indicated Mg was elevated by endrin.

For total serum protein, the values were similar except for the 145-µg/kg group, which was significantly lower than all the others.

Serum cholesterol had a distribution with dose similar to that of serum protein. In groups dosed with 0–14.5 µg/kg, cholesterol and protein tended to increase whereas above 14.5 µg/kg the values decreased.

Trout fed the highest dietary dose of endrin had about 50% more (5 mmoles/liter) serum glucose than was found in exercised controls. All exercised groups had elevated glucose levels compared to the nonexercised controls (Table 1).

The cortisol level in the 4.3-µg/kg group was elevated even more than that of the exercised controls, but those in the higher-dosed groups, 14.5–145 µg/kg, were as low as that of nonexercised controls.

Liver glycogen was directly related to the endrin dose. The amounts in the 4.3- and 14.5-µg/kg groups, however, were not significantly greater than those of the exercised controls.

Variance analysis of the protein amounts in zones I, II, and III of sera electrophoresed on Beckman membranes indicated that endrin affected protein distribution (Table 3). Zones I and III, but not zone II, had greater variance in total protein between parallel zones than within zones of the different groups. Statistical analysis of protein zones resolved on the Brinkman membrane, however, did not indicate significantly greater variances between parallel zones than within parallel zones. The resolution of protein in human control serum on Brinkman and Beckman electrophoretic membranes was similar, but resolution of trout serum proteins was superior on the Brinkman type.

Minor serum constituents and collateral parameters of exercised trout receiving endrin (Table 4) — The minor serum constituents and those that were not affected by endrin are included in this report because they may be reviewed in other studies and a need exists for establishing physiologic "norms" in trout serum composition. We believe also that apparent correlations among the various parameters can be instructive in future studies.

The variance ratios of K and Ca with dose were nonsignificant. The distributions of these electrolytes with respect to dose may correlate, however, with other physiologic parameters, but possible correlations were not tested.

The nonprotein nitrogen fraction varied only slightly among groups and the only statistically significant difference was between the 145- and 14.5-µg/kg groups. Creatinine accounted for a small fraction of the serum NPN. No correlation of

creatinine variation with endrin treatment or exercise was detectable. Also, the serum amino acid level did not correlate with endrin dose. The values varied appreciably and were not significantly different among controls and endrin-dosed trout. The α-amino nitrogen accounted for 22–36% of the NPN in the sera of exercised trout and 68% in that of the nonexercised controls.

Forced swimming elevated serum lactate in all trout, but there was no correlation with endrin dose. The hydrogen ion concentrations of sera were averaged and means were expressed as *p*H. A trend among the different treatment groups may have existed, but it was obscured by a trend related to the time the fish were in the anesthetic. The *p*H estimates were taken rapidly on whole blood without anticoagulant but they dropped progressively (7.35–7.25) during the sampling interval.

Differences in the hepatosomatic index (HSI) were slight and nonsignificant, but a trend in HSI

TABLE 3. Electrophoretic distributions of serum proteins (*g/100 ml*) of trout after 163 days of exposure to various doses of endrin.[a]

Membrane and zone	Dose (μg/kg body wt/day)				
	Control	4.3	14.5	43	145
Brinkman					
I	0.37±0.03[b]	0.48±0.08	0.48±0.05	0.38±0.04	0.30±0.04
II	0.88±0.07	1.00±0.19	0.96±0.09	1.00±0.13	0.80±0.08
III	0.77±0.04	0.86±0.13	0.76±0.04	0.74±0.05	0.66±0.05
IV	0.33±0.02	0.36±0.04	0.40±0.04	0.38±0.04	0.36±0.02
V	0.55±0.05	0.62±0.06	0.56±0.07	0.58±0.05	0.60±0.00
Beckman					
I[c]	1.08±0.04	1.04±0.08	0.74±0.07	0.96±0.07	0.82±0.10
II	0.86±0.04	0.85±0.02	0.76±0.07	0.72±0.05	0.68±0.07
III[c]	1.69±0.08	1.55±0.07	1.61±0.07	1.66±0.07	1.32±0.13

[a]Determinations on 5–7 fish.
[b]Mean and standard error.
[c]Interaction between dose and zonal distribution ($P < 0.05$).

TABLE 4. Minor serum constituents, *p*H, and collateral observations of rainbow trout fed endrin at various rates for 163 days.

	Dose (μg/kg body wt/day)				
	Control (n=11)	4.3 (n=10)	14.5 (n=12)	43.0 (n=10)	145 (n=10)
K (*mEq/liter*)	1.34±0.14[a]	1.52±0.20	2.16±0.32	1.40±0.16	1.54±0.18
Ca (*mEq/liter*)	6.42±0.23	6.14±0.34	6.32±0.18	6.30±0.24	6.02±0.44
NPN (*mg/100 ml*)	21.9±1.4	22.5±1.4	22.4±0.6	22.7±2.0	20.3±0.5
Total α-amino N (leucine equiv. *mmole/liter*)	4.8±1.1	5.8±1.4	3.5±1.1	4.0±1.3	4.8±1.4
Creatinine (*mmole/liter*)	0.141±0.003	0.131±0.003	0.150±0.005	0.137±0.001	0.144±0.008
Lactic acid (*mmole/liter*)	19.9±2.0	23.3±1.6	27.0±2.3	18.2±3.7	27.0±2.6
*p*H	7.225±0.008	7.276±0.009	7.240±0.010	7.249±0.009	7.185±0.009
HSI (%)	1.01±0.03	1.07±0.06	1.10±0.06	0.97±0.05	0.95±0.04
GSI (%)	0.28±0.05	0.32±0.05	0.35±0.04	0.43±0.11	0.26±0.09
Endrin concentration in visceral fat (*μg/kg*)	ND[b] (n=5)	ND (n=5)	760±140 (n=6)	4800±1700 (n=5)	8700 (n=2) (7800 and 9600)

[a]Mean and standard error.
[b]ND < 10 μg/kg.

was similar to that of the serum cholesterol, phosphate, protein, NPN, K, and possibly *p*H (Table 4).

We judged from the gonad–body weight ratios (GSI) that both sexes were in a sexually undeveloped state. Both testes and ovaries were being resorbed in all groups at autopsy.

The endrin concentration in dissected visceral fat varied from no detectable quantity in the control and 4.3-µg/kg groups to 760, 4800, and 8700 µg endrin/kg fat in the 14.5-, 43-, and 145-µg/kg groups, respectively.

Discussion

Forced exercise elicited distinct physiologic adjustments in 9 of the 16 serum parameters examined. Those showing the greatest change, expectedly, were lactate, glucose, amino acids, and cortisol. Because moderate exercise alone is probably not stressful, cortisol increase was likely due, in part, to the fright of our handling the trout, i.e. transferring them from their home tanks to the stamina tunnel, a new environment, and then removing them to anesthetic solution prior to autopsy. Stevens and Black (1966) reported that forced swimming (5 min) of rainbow trout caused muscle lactate to peak immediately and the level in blood to increase more slowly concurrent with lactate release from muscle. Recovery of serum lactate to pre-exercised levels is slow, requiring several hours (Black et al. 1962). In Steven's and Black's studies, no appreciable depletion of liver glycogen occurred after 5 min exercise, but their results suggested mobilization could occur after a longer duration of exercise. Our results are in accord with that contention.

The changes in trout serum and liver composition at autopsy indicate that several physiologic functions were altered by prolonged endrin exposure. However, no clearly defined response could be attributed unequivocally to a primary mode of action. Apparent trends emerged in related physiologic parameters that suggest an endrin-intoxication syndrome.

The major serum electrolytes, Na and Cl, along with serum osmolality, were highly correlated, as expected, and they tended to increase with endrin dosage. We infer from these increases that osmoregulation in rainbow trout was affected either directly or indirectly by endrin. Similar results were obtained with goldfish given the same doses as in the present study. A higher dose (430 µg/kg) in goldfish caused Na and Cl loss and complete osmoregulatory failure (Grant and Mehrle 1970). Serum osmolality of the highest dosed trout increased 4.4% over that of the exercised controls, whereas osmolality of exercised controls increased only 2% above that of nonexercised controls. Differences as great as 4.4% can occur as a normal response to environmental conditions. For example, Holmes and McBean (1963) reported a 9.4% increase in serum Na in rainbow trout on adaptation to salt water and Houston (1959) observed a 4.4% increase in serum Na of rainbow trout in a similar circumstance. Relative changes of 5.7% above the control level were observed in rainbow trout fed dieldrin at concentrations similar to those of endrin in the present study (Grant and Mehrle unpublished). However, in less unusual circumstances than adaptation to sea water or pesticide exposure, regulation of osmolality in a range narrower than 4.4% seems likely, judging from the standard errors of the controls (2 $S_{\bar{x}}$ of exercised controls = 1.6% range; 2 $S_{\bar{x}}$ of nonexercised controls = 0.7% range). Therefore, we assume that endrin toxicosis is characterized by chronic dysfunction of osmoregulatory mechanisms manifested by increased levels of the major electrolytes.

The increased Na and Cl levels relative to endrin dose could not have been caused by increased cell permeability because the concentration gradients existing between tissue and blood would lead to decreased Na and increased K. Eisler and Edmunds (1966) observed increased serum Na and decreased liver concentrations of Na in northern puffers, a marine fish, exposed for 96 hr to near lethal concentrations of endrin. Their data suggest a transfer of Na, but not K, from tissues to blood by some means. Presently, we cannot assess whether the apparently elevated serum Na and Cl concentrations and the concomitant elevation of serum osmolality were deleterious to endrin-treated trout, especially in causing, or resulting from, altered cellular biopotentials as reported symptoms in endrin poisoning suggest. At any rate, Na and Cl retention toward an even higher gradient between blood and fresh water would require a greater energy expenditure and, thus, the loss of intracellular Na would be deleterious, whatever may be the mechanism of efflux. Either intermittent loss, such as during forced-swimming, or sustained low electrolyte concentrations could be harmful to trout by disrupting other homeostatic mechanisms; continual loss would ultimately lead to failure of the organism.

Serum cholesterol, protein, and inorganic phosphate appear to have been interrelated in that they took similar trends with respect to dose and exercise. Even the nonsignificant changes in K and HSI fell in an apparent pattern relative to endrin dosage and exercise. Concentrations (or ratio in the case of HSI) increased in the lower-dosed groups and declined in the higher-dosed groups. A similar

trend with total body lipid was observed in goldfish treated with endrin (Grant and Mehrle 1970). We can only speculate as to the toxicologic significance of these apparent interrelations and trends. Low levels of endrin may actually stimulate certain phys ologic processes, whereas higher dietary doses may cause inhibition or "exhaustion" (as related to an adaptive mechanism). Increased swimming activity in endrin-treated rainbow trout, similar to that in goldfish, suggests that the apparent stimulatory effect of the lower doses may relate to physical conditioning. Definitive estimates of swimming activity could strengthen this contention.

In the case of physiologic parameters assuming a biphasic trend with dosage, two points in the distribution should be considered in describing the minimal dose that caused chronic effects. These points are apparent in the distributions of serum phosphate, protein, cholesterol, and cortisol, and we assume that their increases with the lower doses represent stimulation and the values after inflection represent inhibition. Accordingly, the lowest effective dose represents a potentially harmful dose in our evaluation of toxicologic effects.

Stimulatory effects during the whole life cycle may be ultimately harmful to natural trout populations. That is, "stimulated" populations may be less able than normal populations to interact successfully with the complex ecologic system toward which the species has specialized. Further, low doses of biologically active compounds or external stimuli that stimulate a physiologic process over a relatively brief duration can become inhibitory if administered chronically. Such was the effect of several organochlorine compounds on thyroid activity in several fishes (Grant and Mehrle unpublished).

The points of inflection in dose–response graphs of the above parameters, however, may be interpreted as threshold levels of endrin toxicity. Accordingly, "no effect" dietary intakes may have been between 4.3 and 14.5 µg/kg, based on serum cortisol and serum phosphate values; between 14.5 and 43 µg/kg, based on serum total protein and serum cholesterol; and between 43 and 145 µg/kg, based on the total weight increase.

Liver glycogen decreased significantly in the exercised controls, but not in endrin-treated trout. Liver glycogen in trout fed either 43 or 145 µg/kg was even greater than that of the nonexercised controls. This suggests that glycogenolysis was inhibited by the high endrin concentration, or that gluconeogenesis and glycogenesis were greatly increased, resulting in high glycogen content. Blood glucose also increased in all exercised fish, both control and endrin-treated. However, the increase was not related to endrin dose, except in trout dosed with 145 µg/kg. The latter group of fish exhibited definite hyperglycemia. Correlation of high blood glucose and high liver glycogen in the highest-dosed group suggests further that carbohydrate utilization was impaired.

Cortisol increased significantly in the exercised control group and in the lowest endrin-treated group, but remained similar to the nonexercised control in the three higher-dosage groups. This implies that doses of 14.5 µg/kg endrin and greater are capable of inhibiting interrenal secretion of cortisol, or that these doses stimulate the clearance rate of the hormone. In any case, serum cortisol was the most sensitive parameter to endrin, but its inhibition probably would not have been apparent without our having subjected the dosed fish to exercise.

The accumulation of endrin in the visceral fat ranged from less than 10 µg/kg (fresh fat) in trout dosed with 4.3 µg/kg (body wt)/day to 8700 µg/kg in trout dosed with 145 µg/kg/day. Whole body concentrations of another cyclodiene, dieldrin, were 2.5–3.0% of the concentration in visceral fat (Mehrle and Mayer unpublished).[4] If endrin distributes in the body similar to dieldrin, i.e. whole body concentration/fat concentration = 0.025, estimates of whole body residues are <0.25 µg/kg (body wt), 19.0 µg/kg, 120 µg/kg, and 218 µg/kg for trout receiving doses of 4.3 µg/kg (body wt), 14.5 µg/kg, 43 µg/kg, and 145 µg/kg, respectively. The average whole-body residue of endrin in various fish sampled in the National Monitoring Program (Henderson et al. 1969) was 70 (10–1500) µg/kg. We observed no definite harmful effects of endrin in trout dosed with 4.3 µg/kg/day for 163 days, but we still cannot predict that this intake would be safe indefinitely. On the other hand, we believe the next highest dose, 14.5 µg/kg/day for 163 days, caused harmful effects and represents a definitely unsafe level of dietary intake of endrin. Correspondingly, we believe that trout in natural environments having 19 µg/kg whole-body residues of endrin are receiving unsafe exposures to endrin. This reasoning is probably applicable to most fishes. Endrin residues of 19 µg/kg in fish from the natural environment may be even more detrimental when one considers that endrin residues are complemented with usually much greater amounts of other organochlorine pesticides and industrial pollutants that may act additively or even synergistically.

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[4]Mehrle and Mayer, Fish-Pesticide Research Laboratory, Columbia, Miss. 65201, USA.

Conclusions

Endrin at oral doses of 4.3–145 μg/kg body wt/day for 163 days caused no mortality, but it altered 12 (serum Na, Cl, osmolality, total protein, Mg, PO_4, cortisol, cholesterol, glucose, liver glycogen, growth, and the electrophoretic distribution of serum proteins) of 19 physiologic parameters studied. The sensitivity of the 12 parameters varied with respect to diagnostic usefulness in assessing "adverse effects" and doses that appear to be definitely detrimental to the species. The major physiologic parameters showing effects of endrin were inhibition of glycogenolysis during exercise, serum cortisol suppression, increase of serum osmolality, hyperglycemia, and growth inhibition. Natremia and chloremia may have accompanied the increased serum osmolality. Based on LSDs (least significant differences) the parameters that indicated the lowest effective dose that caused changes from the controls were (1) serum cortisol, inorganic phosphate, Mg, osmolality, and protein (0–4.3 μg/kg); (2) serum cholesterol and liver glycogen (4.3–14.5 μg/kg); and (3) serum glucose and growth (43–145 μg/kg). The distribution of values of HSI, cholesterol, serum protein, inorganic phosphate, NPN, K, and, possibly, *p*H, relative to the dose that the trout received, appeared to be related to endrin toxicosis. The values of the latter were more or less elevated at the intermediate doses and decreased at the higher doses, but we do not know the toxicologic significance of this trend.

Suppression of cortisol secretion (or increased cortisol metabolism) and inhibited carbohydrate metabolism were apparent in trout dosed with 14.5 μg endrin/ kg body wt/day (725 μg/kg food). This effect on a stress–response mechanism implies potentially lowered survival by trout's reduced ability to respond to environmental stresses, such as predation, disease, nutritional deficiency, and physical extremes of temperature and water quality.

Trout and probably other fishes containing 19 μg/kg of endrin (probably less with other organochlorine residues present) very likely are receiving unsafe exposures to endrin.

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Etude du métabolisme de l'eau et de l'azote dans l'hépatopancréas et l'ovaire de *Carcinus maenas*[1]

J.-L. M. MARTIN

Department of the Environment
Fisheries Research Board of Canada, Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

MARTIN, J.-L. M. 1973. Etude du métabolisme de l'eau et de l'azote dans l'hépatopancréas et l'ovaire de *Carcinus maenas*. J. Fish. Res. Board Can. 30: 41–44.

Le présent travail a pour but de déterminer les variations de la teneur en eau, de l'azote protéique et soluble au cours du cycle d'intermue et de l'ovogénèse respectivement dans l'hépatopancréas et l'ovaire de *Carcinus maenas*. Les analyses relatives à l'hépatopancréas font ressortir trois causes de variations: l'entrée de l'eau dans l'organisme pendant et après l'exuviation, entraînant une dilution des éléments étudiés, les périodes de jeûne et de nutrition au cours du cycle d'intermue, et l'ovogénèse qui, nécessitant de grandes quantités de matériel azoté pour atteindre son terme, sera la cause d'une baisse des taux d'azote dans l'hépatopancréas. Des variations dans le métabolisme de l'eau et de l'azote sont mises en évidence dans l'ovaire au cours de l'ovogénèse.

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Measurements were made of variations in water, protein, and soluble nitrogen in the hepatopancreas of *Carcinus maenas* during the intermolt cycle and in the ovary during ovogenesis. Analyses of hepatopancreas show three reasons for the variations in protein and soluble nitrogen contents: entry of water into the organism during and after exuviation and a corresponding dilution of the constituents, alternation of fasting and feeding during the intermolt cycle, and heavy demands of ovogenesis resulting in a decrease in nitrogen in the hepatopancreas. Variations in the metabolism of water and nitrogen are shown in the ovary during ovogenesis.

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LA croissance des Crustacés décapodes est conditionnée par le phénomène de la mue. Celui-ci, se reproduisant de façon cyclique à intervalles de temps variables selon la taille et l'âge de l'animal, ne doit être considéré que comme l'aboutissement d'une suite de transformations physiologiques et morphologiques se produisant dans l'organisme de l'animal (Drach 1939; Passano 1960).

L'hépatopancréas (ou glande digestive) qui est le siège des fonctions inhérentes au métabolisme alimentaire verra sa physiologie varier au cours des différentes étapes du cycle d'intermue. De même, au cours de l'ovogénèse, l'ovaire subira des modifications d'ordre physiologique et biochimique parallèlement aux variations d'ordre anatomique: augmentation de la taille, changement de coloration.

Le présent travail portera sur les variations de la teneur en eau, de l'azote protéique et soluble au cours respectivement des deux phénomènes précités: cycle d'intermue et ovogénèse.

Matériel et Méthodes

Les crabes capturés près des côtes bretonnes, dans la région de Roscoff, sont transferrés dans un grand bac rectangulaire alimenté en eau de mer courante, bien aérée à l'aide de diffuseurs d'air. Ils sont nourris régulièrement avec de la chair de poissons et de crevettes. Les animaux montrant les signes morphologiques d'une mue proche, ainsi que ceux ayant récemment mué, sont isolés individuellement dans des bacs en polyéthylène. Les crabes blessés, sexuellement immatures ou présentant des traces de parasitisme sont éliminés de notre étude.

Les étapes du cycle d'intermue sont déterminées par la méthode de Drach (1939) et Drach et Tchernigovtzeff (1967). Les organes sont prélevés selon la méthode préconisée par Renaud (1949).

L'organe, après prélèvement et pesée précise, est broyé dans 5 à 10 ml d'acide trichloracétique (TCA) à 10% pendant une heure, et homogénéisé de temps en temps

[1]Ce travail fait partie d'une thèse de spécialité présentée le 28 Octobre 1969, à l'Université de Marseille, France.

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avec un petit agitateur en verre. Le mélange est alors versé sur un filtre exempt d'azote, à filtration lente, où il décante. Le TCA contenant l'azote soluble est recueilli. Le résidu protéique retenu sur le filtre est lavé à plusieurs reprises par du TCA à 10%. Ces opérations sont effectuées à la température de 0 C.

L'azote est minéralisé dans de l'acide sulfurique concentré et pur. Le catalyseur employé est formé d'un volume de sélénium pour 60 volumes de sulfate de potassium.

Les dosages sont effectués selon la méthode de Nessler modifiée par Strauch (1965). La gamme étalon est établie à partir d'une solution titrée de sulfate d'ammonium. La densité optique des échantillons et de la gamme étalon est mesurée à une longueur d'onde de 490 nm au spectrophotomètre.

Résultats

Hepatopancreas

Après l'exuviation et jusqu'au stade B_1 B_2 la teneur en eau de l'organe augmente jusqu'à 80% du poids frais de celui-ci (Fig. 1). Du stade B_1 B_2 jusqu'à la mue, ces valeurs decroîtront. Les variations observées de A_1 A_2 à B_1 B_2 et de B_1 B_2 à D_3 D_4 sont significatives ($P < 0.01$).

L'azote protéique et l'azote soluble montrent des variations sensiblement identiques entre elles (Fig. 1). En fonction du poids frais de l'organe, après une baisse succédant à la mue, nous observons une croissance des taux d'azote à partir de C_1 C_2. Les valeurs passent par un maximum en D_1 D_2, puis jusqu'à l'exuviation elles diminuent. A partir du stade C_4, et jusqu'à l'exuviation, le rapport $\frac{\text{azote soluble}}{\text{azote protéique}}$ est supérieur à 1.

Les variations observées, pour l'azote protéique et pour l'azote soluble, entre les stades B_1 B_2 et D_1 D_2 sont significatives ($P < 0.01$). Ces stades correspondent respectivement à la fin de la période de jeûne et à la fin de la période de nutrition par lesquelles l'animal passe au cours du cycle d'intermue.

La courbe de variations de l'azote protéique en fonction du poids sec (Fig. 1) présente deux maximums situés en C_1 C_2 et D_1 D_2. Ces deux maximums sont séparés par un taux minimal d'azote en C_4. La décroissance observée de C_1 C_2 à C_4 est significative ($P < 0.05$).

Ovaire

Les variations de la teneur en eau (Fig. 2) exprimées en fonction du rapport gonado-somatique R[2] peuvent être exprimées par une courbe de type

[2] $R = \frac{\text{Poids frais de l'ovaire}}{\text{Poids de l'animal}} \times 100.$

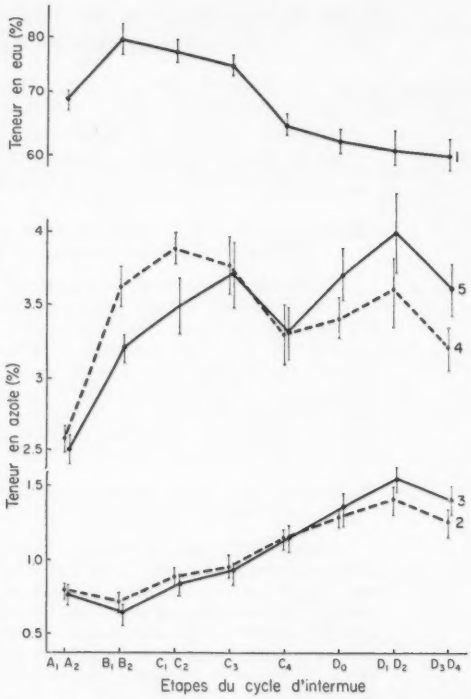

FIG. 1. Hépatopancréas. Variations au cours du cycle d'intermue de la teneur en eau (1), des taux d'azote protéique (2) et soluble (3) rapportés au poids frais de l'organe, des taux d'azote protéique (4) et soluble (5) rapportés au poids sec de l'organe.

$y = ax^b$. Cette courbe ($r: 0.851$; $P < 0.01$) témoigne d'une décroissance rapide de la teneur en eau au début de l'ovogénèse, suivie d'une stabilisation lorsque la maturation de l'ovaire tend vers son terme.

Les variations des taux d'azote protéique et soluble en fonction du poids frais de l'organe (Fig. 2) montrent au début de la maturation de l'ovaire une croissance jusqu'à une valeur approximative de $R = 3$. Les taux d'azote protéique et soluble sont corrélés aux valeurs de R ($P < 0.01$.) Les pentes de ces deux courbes de régression sont significativement différentes ($P < 0.01$) et témoignent d'une accumulation plus intense d'azote protéique que d'azote soluble pour $R < 3$.

Pour les valeurs, de $R > 3$ les taux d'azote protéique et soluble décroissent. Ceux-ci sont corrélés aux valeurs de R($P < 0.01$). Les taux d'azote protéique décroissent plus rapidement que les taux d'azote soluble.

Les variations des quantités d'azote protéique et soluble, rapportées à un animal de 100 g, présentes dans l'ovaire au cours de l'ovogénèse peuvent être exprimées par deux droites de régression (Fig. 3). Les taux d'azote protéique et soluble corrélés aux valeurs de R($P < 0.01$) croissent tout au long de l'ovogénèse. Nous remarquerons toutefois que cette croissance semble s'effectuer d'une façon plus rapide pour des valeurs de $R < 3$.

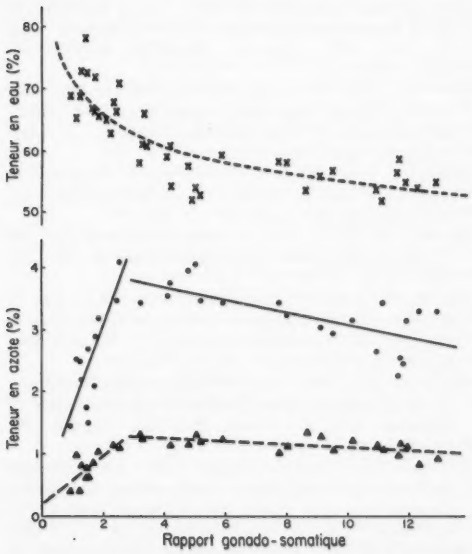

FIG. 2. Ovaire. Variations de la teneur en eau (x), des taux d'azote protéique (●) et soluble (▲) rapportés au poids frais de l'organe au cours de l'ovogénèse.

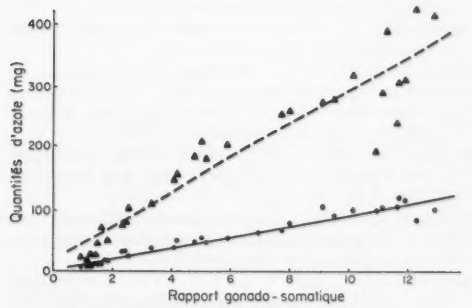

FIG. 3. Ovaire. Variations des quantités d'azote protéique (▲) et soluble (●) contenus dans l'ovaire, pour un animal de 100 g, au cours de l'ovogénèse.

Discussion

L'étude des variations des taux d'azote protéique et soluble dans l'hépatopancréas nous montre que celles-ci sont sous la dépendance des fluctuations de la teneur en eau ainsi que des périodes de jeûne et de nutrition. Du stade A_1 A_2 au stade B_1 B_2 les taux d'azote baissent. Ces mêmes taux augmentent si nous les rapportons au poids sec de l'organe. Dans cette partie du cycle d'intermue les fluctuations de la teneur en eau jouent un rôle prépondérant sur les variations des taux d'azote. Durant les stades D_1 D_2 et D_3 D_4 les taux diminuent en même temps que la teneur en eau. La baisse observée ne peut être imputable dans ce cas précis qu'au jeûne de l'animal et à la consommation des réserves.

En fonction du poids sec de l'organe les taux d'azote croissent de A_1 A_2 à C_1 C_2. Il y a tout lieu de supposer que l'importance prise par les éléments azotés est essentiellement due à l'azote de constitution des tissus de l'hépatopancréas et à une décroissance des taux des éléments non azotés de l'organe. L'animal terminant en C_1 C_2 sa période de jeûne a un hépatopancréas en principe très pauvre en matériel de réserve. Renaud (1949) met en évidence chez *Cancer pagurus* une baisse des taux de lipides et de glycogène du stade A_1 au stade C_2. Des observations comparables sont effectuées par Heath et Barnes (1970) chez *C. maenas*. D'autre part, Glynn (1968) montre, chez *Homarus vulgaris*, une baisse des taux des minéraux, calcium et magnésium au cours des stades succédant à la mue.

Les variations des taux d'azote protéique en fonction du poids sec de l'hépatopancréas coïncident sensiblement avec celles effectuées chez *C. pagurus* par Renaud (1949), qui a obtenu une courbe de variations unimodale. Renaud opère sur des animaux immatures pour lesquels il n'y a pas d'utilisation de matériel azoté pour l'édification des tissus de l'ovaire et la maturation des gamètes. Les quantités d'azote protéique contenu dans l'ovaire parvenu à maturité et dans l'hépatopancréas d'un animal de 100 g au stade C_4 sont respectivement estimées à 400 et 45 mg. D'autre part, le minimum observé au stade C_4 coïncide avec un maximum des taux de protéines et de caroténoïdes sériques (Martin et Ceccaldi 1969) en même temps que dans l'ovaire se produit une accumulation de caroténoprotéines de couleur orange (Martin 1972) responsables de la coloration de l'ovaire. Abeloos et Fisher (1926) notent une décoloration de l'hépatopancréas au cours de l'ovogénèse.

Il semble donc qu'au cours du stade C_4, d'importantes quantités d'azote, sous forme de protéines et de caroténoprotéines soient prélevées dans l'hépatopancréas et accumulées dans l'ovaire pour per-

mettre la réalisation de l'ovogénèse. Ces faits justifient les taux minimaux d'azote observés au stade C_4.

De grandes quantités d'azote soluble sont mises en jeu dans l'hépatopancréas durant tout le cycle d'intermue et plus particulièrement pendant la période d'alimentation active de l'animal. A partir du stade C_4 les taux d'azote soluble sont supérieurs à ceux observés pour l'azote protéique. Ces résultats témoignent d'un métabolisme intense au niveau de l'organe. Bauchau et Mengeot (1965) notent au cours des stades C_4 à D_1 un forte activité enzymatique alors que celle-ci est moindre pendant la période de jeûne. Van Wormhoudt et al. (1972) montrent chez *Palaemon serratus* que l'activité protéasique dans l'hépatopancréas est maximale pour les stades C et D_2 D_3. Il est permis de penser que l'azote soluble est issu, pour sa plus grande part, du métabolisme de transformation ou de synthèse des protéines. Cet azote soluble sera sécrété en partie sous forme d'ammoniaque au moment de la mue (Needham 1956) où, combiné au glycogène, il donnera l'acétylglucosamine dont la polymérisation des molécules fournira la chitine (Renaud 1949).

L'observation des courbes de variation des taux d'azote dans l'ovaire mettent en évidence deux phases correspondant respectivement à des valeurs de R inférieures à 3 ou supérieures à cette valeur. Il semblerait que l'ovaire subisse à partir de cette valeur de R une modification dans son métabolisme azoté, ou du moins dans l'intensité de ce dernier.

L'étude des variations des quantités totales d'azote nous permet de faire deux remarques: d'une part l'ovaire est, durant toute l'ovogénèse, l'objet d'un apport d'azote. Cet apport a la possibilité de s'effectuer à travers la membrane vitelline dont la perméabilité a été démontrée par Cheung (1966). D'autre part, l'ovogénèse nécessite, pour parvenir à son terme, l'apport de grandes quantités d'azote.

Les variations de l'azote soluble montrent de remarquables analogies avec celles de l'azote protéique. Il est permis de penser que, comme dans le cas de l'hépatopancréas, l'azote soluble est issu pour sa plus grande part du métabolisme de transformation ou de synthèse des protéines.

Les résultats obtenus seront ultérieurement complétés par une étude des variations d'éléments tels que le glycogène, les lipides et les minéraux de constitution. De même nous nous attacherons à démontrer les rapports qui peuvent exister entre l'intensité du métabolisme azoté chez les Crustacés décapodes et la composition biochimique, qualitative et quantitative de la nourriture ingérée par l'animal. Les recherches effectuées dans cette voie, tant sur les vertébrés (Beerstecher 1963) que sur les invertébrés planctoniques (Corner et Cowey 1968), montrent que la teneur en azote et son métabolisme varient en fonction du régime alimentaire fourni à l'animal.

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Hexose Monophosphate Accumulation and Related Metabolic Changes in Unfrozen and Thawed Adductor Muscle of the Sea Scallop (*Placopecten magellanicus*)

DORIS FRASER HILTZ AND W. J. DYER

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

HILTZ, D. F., AND W. J. DYER. 1973. Hexose monophosphate accumulation and related metabolic changes in unfrozen and thawed adductor muscle of the sea scallop (*Placopecten magellanicus*). J. Fish. Res. Board Can. 30: 45–52.

The hexose monophosphates (glucose-6-phosphate (G_6P), fructose-6-phosphate, and glucose-1-phosphate) accumulate postmortem in scallop adductor muscle (meats) during chill storage, increasing from only traces at death to levels totalling about 10 μmole/g after 8–10 days. In both unfrozen and thawed meats, G_6P accounted consistently for 80–85% of the total hexose monophosphate, over a wide range of actual concentrations. Quick freezing at any time postmortem did not affect the concentrations of hexose monophosphates or related metabolic constituents. Thawing of the prerigor-frozen muscle, however, either slowly or rapidly, resulted in considerable G_6P formation, and accelerated markedly the subsequent rate and extent of G_6P production. The exceptionally high levels (~20 μmole/g) reached in the thawed meats after 1–2 days at 5 C are implicated in the development of burned flavors, odors, and discolorations (i.e. Maillard browning reactions) observed in these thawed meats when cooked. While thawing of the prerigor-frozen muscle led to some hypoxanthine (Hx) and octopine formation, the subsequent rate of Hx accumulation at 5 C was three times faster in the thawed meats than in unfrozen meats. In meats frozen postrigor, thawing resulted in negligible change in concentrations of the metabolites; subsequent patterns of change during storage differed little from those in unfrozen muscle, although Hx accumulation was more rapid. Hx content showed potential as an index of quality in thawed scallop muscle.

HILTZ, D. F., AND W. J. DYER. 1973. Hexose monophosphate accumulation and related metabolic changes in unfrozen and thawed adductor muscle of the sea scallop (*Placopecten magellanicus*). J. Fish. Res. Board Can. 30: 45–52.

Il y a accumulation post-mortem d'hexoses monophosphates (glucose-6-phosphate (G_6P), fructose-6-phosphate et glucose-1-phosphate) dans le muscle adducteur (chair) de la pétoncle durant l'entreposage à froid, sur une gamme allant de traces seulement au moment de la mort à des niveaux atteignant environ 10 μmoles/g 8 à 10 jours après. Dans les chairs non congelées tout comme dans celles décongelées, le G_6P constitute invariablement 80 à 85% des hexoses monophosphates, avec gamme étendue d'actuelles concentrations. La congélation rapide, n'importe quand après la mort, n'affecte pas la teneur des hexoses monophosphates ou celle des constituants métaboliques connexes. Cependant, la décongélation lente ou rapide du muscle congelé avant l'état de rigor a pour résultat la formation d'une grande quantité de G_6P et subséquemment l'augmentation du taux et de l'étendue de production du G_6P. Les niveaux exceptionnellement hauts (~ 20 μmoles/g) atteints par les chairs décongelées après 1 ou 2 jours à 5 C y sont pour quelque chose dans le développement, à la cuisson, d'un goût, odeur et couleur de brûlé (i.e. les réactions de brunissement de Maillard). Alors que la décongélation des chairs congelées avant la rigor cause la formation d'hypoxanthine (Hx) et d'octopine, le taux subséquent d'accumulation de Hx à 5 C est trois fois plus grand dans les chairs décongelées que dans celles non congelées. La décongélation de chairs congelées après l'état de rigor ne cause que des changements mineurs de concentration des métabolites; les changements subséquents au cours de l'entreposage ne diffèrent que très peu de ceux observés dans les muscles non congelés, bien que l'accumulation de Hx soit plus rapide. La teneur en Hx offre des possibilités comme norme de qualité des chairs de pétoncle décongelées.

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PREVIOUS studies in this laboratory (Hiltz and Dyer 1970, 1971) have shown that postmortem metabolism in scallop adductor muscle is marked by two major biochemical changes: accumulation of Hx[1], arising from the dephosphorylation and deamination of mononucleotide precursors; and production of octopine, as a principal end-product of the concomitant glycogen degradation. We now extend these findings to include first, the intermediate sugar phosphate metabolites of the glycolytic cycle, particularly the hexose monophosphates, and secondly, the effects of freezing and thawing on the subsequent enzyme-catalyzed glycolytic and nucleotide degradative processes in scallop meats which had been frozen both pre- and postrigor.

Freezing and thawing may alter enzymic activities drastically (Chilson et al. 1965; Tappel 1966). A well-documented manifestation of this effect is "thaw rigor," whereby activation of the myofibrillar ATPase during thawing of prerigor-frozen muscle results in a very rapid and complete loss of the high-energy phosphate compounds ATP and creatine phosphate (e.g. Bendall 1960; Partmann 1969). The observed increase in activity of malic enzyme (Gould 1968) and of glutamic-oxaloacetate transaminase (Hamm and Körmendy 1969) in the sarcoplasm effected by freezing and thawing has been the basis for reliable differentiation between unfrozen and frozen-and-thawed fish and mammalian muscle. Our interest in the effects of the freeze–thaw process on the rate and extent of the major metabolic changes in scallop muscle was stimulated by the observation that a considerable proportion of the "fresh" scallop meats marketed locally are in reality frozen meats thawed prior to sale.

Several metabolic intermediates and end-products of glycolysis and nucleotide degradation are known to contribute significantly to flavor and quality changes in seafoods (see reviews by Tarr 1966, and Jones 1967); studies have been confined chiefly to fish, with marine invertebrate forms receiving comparatively little attention. Of particular interest are the "sugar phosphates" (hexose and pentose phosphates), which have been implicated in Maillard browning reactions in heated and dried fish muscle (Burt and Jones 1961; Tarr 1965). The contribution of the mononucleotides to flavor is well established and their principal degradation product, Hx, provides a useful index of storage time and of eating quality for several fish species (e.g. Burt et al. 1969). We previously noted the potentiality of Hx content as a quality index in scallop meats during iced storage, where flavor scores correlated well with concentrations of the purine (Hiltz and Dyer 1970). The present work confirms and extends these findings to storage of scallop meats at +5 C (approximating retail and household cabinet temperatures), and to thawed meats. The results also indicate that the extensive metabolic changes brought about by thawing may affect markedly the quality of the thawed product and thus are of considerable technological importance.

[1]The following abbreviations are used: Hx, hypoxanthine; Ino, inosine; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; IMP, inosine 5′-monophosphate; AP, arginine phosphate; G_6P, D-glucose-6-phosphate; F_6P, D-fructose-6-phosphate; G_1P, α-D-glucose-1-phosphate; NAD, nicotinamide adenine dinucleotide; NADP, $NADPH_2$, oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate, respectively.

Materials and Methods

Procedures used in the handling and processing of live scallops from the Bay of Fundy or Northumberland Strait inshore fisheries, postmortem storage of the adductor muscles (meats) at 0 or 5 C, composite sampling, and extraction of metabolic constituents with 6% perchloric acid have been described previously (Hiltz and Dyer 1970).

When required, meats were individually quick-frozen in a vertical plate freezer (plate temperature –40 C), packaged six meats to a polyethylene bag, and stored at –26 C for no more than 9 weeks before thawing. Thawing was accomplished either slowly, by placing the bags containing the meats in a closed cardboard carton in still air at 2 C overnight (17 hr), or rapidly, by immersion of the bags in water at 20 C for 1 hr. Times to pass through the critical zone from –5 to –0.8 C, measured with thermocouples, averaged 8 hr and 7 min for slowly and rapidly thawed meats, respectively. If required, the thawed meats were then transferred to fresh polyethylene bags for subsequent storage at 5 C. There was no noticeable drip on thawing.

Procedures for determination of *p*H, AP, ATP, glycogen, Hx, and octopine; for thin-layer chromatography; and for organoleptic examination and taste testing of the steamed meats were those used previously (Hiltz and Dyer 1970, 1971). The hexose monophosphates, G_6P, F_6P and G_1P, were measured by a combined procedure based on methods described by Hohorst (1963) and Bergmeyer and Klotzsch (1963); the relevant enzymes were added sequentially. The assay mixtures contained triethanolamine buffer 0.2 M at *p*H 7.6, $MgCl_2$ 5×10^{-3} M, NADP 2.6×10^{-4} M, neutralized perchloric acid extract, and enzymes in a total volume of 3.0 ml; resultant formation of $NADPH_2$ was measured at 340 nm in a Beckmann spectrophotometer.

Results

We found that G_6P consistently accounted for 80–85% of the total hexose monophosphate over the wide range of actual concentrations encountered

during unfrozen storage, thawing, and storage of the thawed meats. Values for the ratio of G_6P to F_6P fell mostly in the range 4.0–5.0, similar to those reported for mammalian (ox) muscle (Newbold and Scopes 1967) and for fish (cod) muscle (Burt 1961). Only relatively low concentrations of G_1P were measured at any time postmortem; maximum levels attained were about 0.5 μmole/g (5–6% of the total hexose monophosphate) after 12–16 days storage.

Changes in Hexose Monophosphate Concentrations During Postmortem Storage of Unfrozen Scallop Meats

During storage of unfrozen meats at 0 or 5 C (Fig. 1a and 3a), hexose monophosphate concentrations (represented by G_6P, the predominant phosphate) increased gradually from only traces at death to levels of 2–3 μmole/g at the onset of rigor (4–6 days); the rate of accumulation then increased sharply, with levels rising to 8–10 μmole/g at 8–10 days.

Metabolic Changes upon Thawing of Scallop Meats Quick-Frozen after 0–16 Days in Ice

Quick freezing itself, and frozen storage for up to 9 weeks at −26 C, did not produce any alteration in the concentrations of the metabolites, as noted previously with respect to octopine content (Hiltz and Dyer 1971); levels of glycogen, glycolytic intermediates, octopine, nucleotides, and their degradation products were unchanged from those in unfrozen meats at any time postmortem ranging from 0 to 16 days.

Thawing overnight at 2 C, however, produced striking and extensive changes in concentrations of metabolites that were confined principally to muscles frozen prerigor (up to 4–6 days in ice) (Fig. 1). G_6P content showed exceptionally large increases (Fig. 1a), with levels on thawing approximating those attained in unfrozen meats only after 10–16 days in ice (7–10 μmole/g). The extent of change was less in meats frozen near the onset of rigor. Changes in octopine content on thawing were less marked (Fig. 1b), although increases of 5–9 μmole/g were measured in meats frozen within 2 days of shucking. Small decreases in *p*H (0.1–0.2 units, from prethaw values of 6.8–7.0) accompanied the octopine formation. Glycogen content decreased on thawing by 11–17 μmole/g (as glucose), from levels of 90 μmole/g (~1.6%) in the frozen meats, a loss approximately equivalent to the combined accumulation of hexose monophosphate and octopine. The quick-frozen prerigor meats

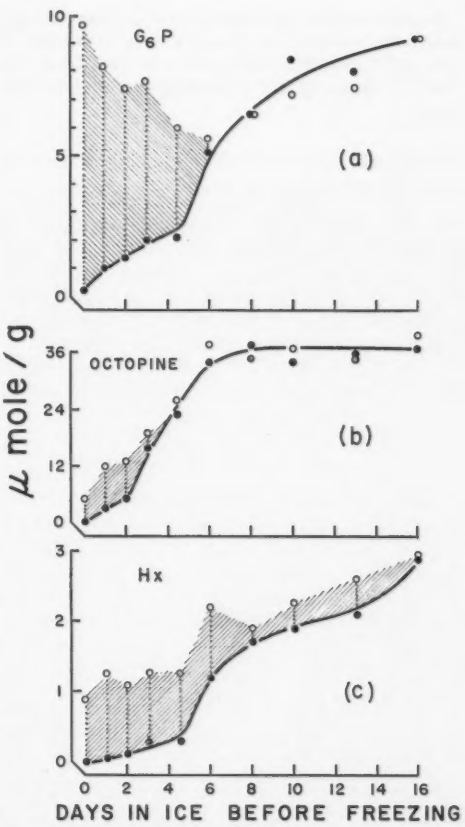

FIG. 1. Changes in G_6P, octopine, and Hx upon thawing (overnight at 2 C) of scallop meats quick-frozen after various periods of iced storage. Metabolic contents in the quick-frozen meats (unchanged from levels in the iced meats) are given by the solid circles; the line joining them shows the change in the unfrozen meats with storage time. Levels of metabolites upon thawing are represented by the open circles, with the extent of change on thawing indicated by the dotted line joining the points of each pair (shaded area). Each point represents a composite sampling of 4–6 scallop meats.

contained appreciable amounts of high-energy phosphate compounds, up to 30 and 6 μmole/g of AP and ATP, respectively; thawing resulted in complete hydrolysis of AP, and in dephosphorylation of ATP to AMP (Fig. 2). It also resulted in further degradation to Hx, since Hx levels in the thawed meats frozen pre- and in-rigor (up to 6 days) were about 1 μmole/g higher than in the iced or quick-frozen meats (Fig. 1c).

In meats frozen postrigor (after 6–16 days storage), metabolic changes on thawing were negligible in contrast to meats frozen prerigor, except for Hx, where increases up to 0.5 μmole/g occurred in some cases.

Metabolic Changes During Subsequent Storage of Thawed Meats at 5 C

In this study, scallop meats frozen both at the prerigor stage (iced 1 day) and at the postrigor stage (iced 6 days) were thawed both slowly and rapidly (8 hr and 7 min, respectively, in the critical zone) and the thawed meats were stored at 5 C, simulating conditions likely to be encountered in retail practice. Unfrozen meats stored at 5 C from the time of shucking, as well as others transferred to 5 C after 1 day and 6 days in ice, served as controls.

Prerigor

Thawing of the prerigor-frozen muscle again resulted in significant increases in content of G_6P, octopine, and Hx (Fig. 3a, b, c), and complete hydrolysis of AP and ATP. In the meats thawed slowly, somewhat higher levels of G_6P and Hx were reached than in those thawed rapidly, but this probably reflects the longer time elapsed before first sampling of the slowly thawed meats (thawed overnight).

On subsequent storage at 5 C, hexose monophosphate concentrations in the prerigor-frozen-and-thawed meats continued to rise sharply from the elevated levels measured on thawing (Fig. 3a), reaching peaks close to 20 μmole/g after 1–2 days storage, and thereafter decreasing more slowly to levels similar to those ultimately reached in the control meats. The pattern of change was similar with both thawing conditions, although peak levels were achieved somewhat earlier in the slowly thawed meats. Although octopine contents rose from 5 to 14–15 μmole/g on thawing, the subsequent rate of development was relatively slow (Fig. 3b), particularly in the rapidly thawed meats, where it was much slower than that of the unfrozen controls. After an initial *p*H drop of 0.2–0.3 units on thawing, the rate of *p*H decrease on subsequent storage was slow, concomitant with the slow octopine production. We found that losses in glycogen content were proportional to the amounts of hexose monophosphate and octopine formed, both on thawing, as in the previous lot of meats, and up to 2 days' storage; thereafter glycogen decreases were greater, indicating the possibility that other pathways of glycogen degradation may exist or other intermediates accumulate in the thawed meats (see Hiltz and Dyer 1971). The rate of Hx accumulation in the prerigor-thawed meats was extremely rapid, about three times that of the control meats (Fig. 3c). The very high levels ultimately reached, 5.6–5.7 μmole/g, which were over twice those attained in the control meats even after 16 days' storage, approached the ATP values measured at death (6.1 μmole/g), indicating a stoichiometric conversion of ATP through the degradative sequence of reactions to Hx.

Postrigor

Thawing of postrigor meats (frozen after 6 days in ice) resulted, as previously, in negligible change in concentrations of metabolites (Fig. 3d, e, f). Subsequent changes in G_6P and octopine patterns with storage of the thawed meats at 5 C did not differ significantly from those of the control meats between 6 and 16 days. Hx production, however, continued to increase at a much faster rate than that of the control meats, and, again, the ultimate levels reached were about twice as high.

Curiously, in the unfrozen control meats, the initial rates of ATP degradation, and of G_6P and Hx accumulation (Fig. 3d, f) from 0–6 days postmortem were faster in the iced meats than in those held at 5 C. These unexpected results are similar to those reported for ox muscle (Newbold and Scopes 1967), in which glycolytic and nucleotide degradative rates appeared faster at 1 C than at 5 C.

Discussion

Our findings indicate that accumulation of hexose monophosphate, of which at least 80% was found to be G_6P, is one of the major metabolic processes occurring in scallop muscle postmortem. Any significant accumulation appears to be delayed until octopine production is at or near its maximum, indicating a rapid glycolytic flux throughout the Embden–Meyerhof pathway from glycogen to pyruvate and thence to octopine during the early part of the storage period. The sharp increase in G_6P concentrations following maximum octopine accumulation (Fig. 1a, b) suggests a slowing or cessation of phosphofructokinase activity, due possibly to the fall in muscle *p*H (Danforth 1965) to ultimate levels of 6.0–6.2 and to the absence of ATP at this time. The fact that fructose diphosphate and triose phosphate, measured enzymically according to Bücher and Hohorst (1963), were not found in concentrations greater than 0.06 and 0.07 μmole/g respectively in either unfrozen or thawed scallop muscle at any time postmortem, indicates that a block is not likely to occur at the aldolase or triosephosphate dehydrogenase steps. It appears that accumulation of hexose monophosphate postmortem may be characteristic of *Pecten* sp., since substantial increases in "hexose

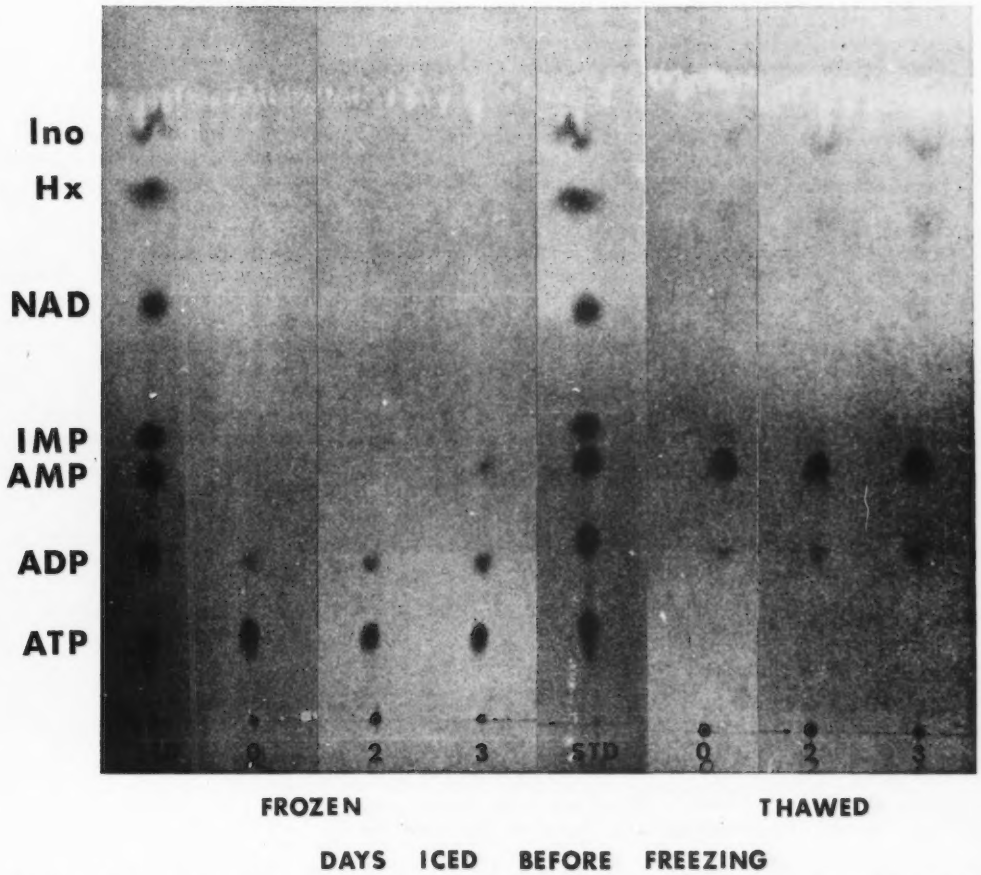

FIG. 2. Thin-layer chromatogram showing the nucleotide degradation that occurs when scallop meats frozen pre-rigor are thawed.

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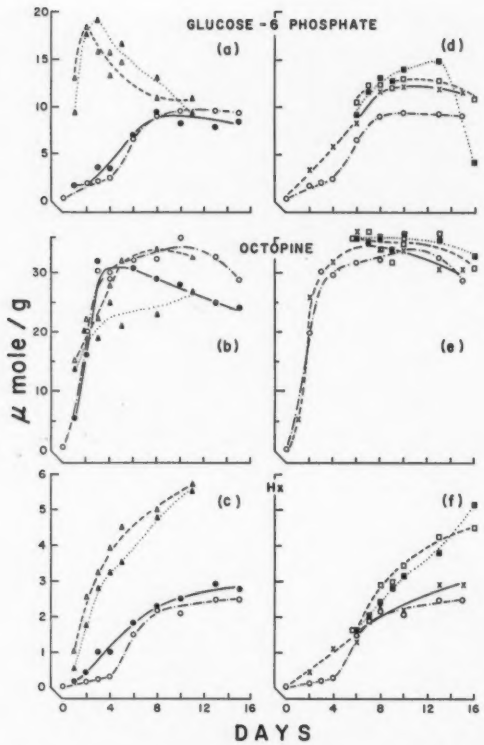

FIG. 3. Comparative changes in G_6P, octopine, and Hx during storage at 5 C in unfrozen meats and in thawed meats which had been frozen prerigor (a, b, c) and postrigor (d, e, f). The unfrozen meats were stored continuously at 5 C (○); iced 1 day, then stored at 5 C (●); iced 6 days, then stored at 5 C (X). The meats frozen prerigor (iced 1 day) were thawed rapidly (▲) and slowly (△) before storage at 5 C. The meats frozen postrigor (iced 6 days) were similarly thawed rapidly (■) and slowly (□) before storage.

phosphate" content during iced storage have also been reported for meats of the weathervane scallop, *Platinopecten caurinus* (Groninger and Brandt 1970). In unfrozen muscle of fish, on the other hand, the pattern of change is somewhat different. In aquarium (unexercised) cod, G_6P levels in the iced muscle rose to 5–6 μmole/g at 4 days, then fell to < 1 μmole at 10 days (Burt 1961); in trawled cod, G_6P levels were highest at death, 2.2 μmole/g, and decreased abruptly thereafter (Burt and Jones 1961). These changes probably reflect the faster overall catabolic rate in fish muscle compared to that in invertebrate (scallop) muscle. Extreme variations (0.2–11 μmole/g) in G_6P content of the muscles of various fish species, pre- and postmortem history largely unknown, have been reported by Tarr and Leroux (1962).

The process of freezing and thawing dramatically accelerated the rates of some major glycolytic and nucleotide degradative reactions in postmortem scallop adductor muscle, particularly in that frozen prerigor. Presumably, the disruption in cellular structure brought about by the freeze–thaw cycle led to activation of the relevant enzymes, perhaps as a result of increased availability of substrate to enzyme or release of enzymes from cell organelles. The accumulation of large amounts of G_6P on thawing, and its continued increase to peak values within 2 days of storage of the thawed material, suggests that phosphorylase was activated to a greater extent than phosphofructokinase. This was also apparent upon thawing of thin strips of ox muscle (Scopes and Newbold 1968), and may be due to the stimulation of phosphorylase *b* into the active *a* form reported to occur upon freezing of prerigor muscle (Cori 1956). In the rapidly thawed muscle (Fig. 3a, b), the rate of accumulation of octopine was slower than the decrease in G_6P from 2 to 10 days postthawing, suggesting that some step(s) beyond phosphofructokinase became rate-limiting for octopine production in the thawed muscle. Alternatively, G_6P may possibly be metabolized via the hexose monophosphate shunt system, although it is not known to what extent this pathway is operative in scallop muscle. In the postrigor muscle, the relative stability of G_6P levels on thawing, even though considerable glycogen remained at this time (1.9%), indicates a lack of stimulation of phosphorylase, possibly due to the low muscle *p*H (6.1–6.2), which is known to inhibit phosphorylase activity (Cori 1956; Danforth 1965). The observation that the decrease in muscle *p*H on thawing of prerigor muscle, and also on subsequent storage, was proportional to the octopine accumulation agrees with our previous finding of a linear relation between octopine production and *p*H fall in ice-stored meats (Hiltz and Dyer 1971).

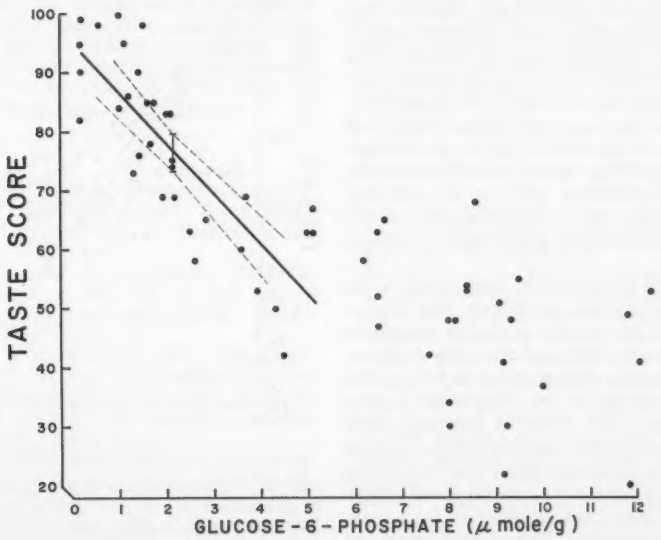

FIG. 4. Regression line of taste scores on G_6P contents (up to 5 μmole/g) in several lots of unfrozen scallop meats stored at 0 or 5 C. 95% confidence limits are shown; Y = 95–8.5 X (n = 33), where Y is taste score and X is G_6P content (μmole/g).

of the nucleotide degradative enzymes appears to be much greater in scallop muscle than in fish muscle. Jones (1965) reported that IMP actually increased on slow thawing of postrigor cod muscle, indicating little or no stimulation of the 5′-nucleotidase (IMP-phosphohydrolase) activity. Rates of IMP degradation in various Pacific coast fish species (Kemp and Spinelli 1969) and of Hx accumulation in freshwater species (Dugal 1967) did not differ significantly after thawing from those in the unfrozen muscle. In thawed mackerel, rate of Hx accumulation was somewhat faster than that in unfrozen controls (Fraser et al. 1968).

The usefulness of G_6P content as an index of quality in unfrozen scallop meats seems confined to the early storage period, as confirmation of freshness, even though appreciable amounts of G_6P accumulate within the period of edibility. A regression line of flavor scores on G_6P contents (Fig. 4) showed a highly significant ($P < 0.001$) linear relation up to G_6P levels of about 5 μmole/g, corresponding to taste scores of 50–55, well above the point of unacceptability (score of 40); at higher G_6P levels, both G_6P content and taste scores varied widely. In the thawed meats, the sharp fluctuation in G_6P content on subsequent storage (Fig. 3a) precluded its use as a quality index. On the other hand, Hx content may have potential in this respect, since increase in Hx content on storage of the thawed muscle appears to be linearly related to decrease in taste score (Fig. 5). In unfrozen muscle, there is a highly significant relation between taste score and Hx content (Fig. 5), in agreement with that previously established (Hiltz and Dyer 1970). The lesser slope of the regression line for the thawed meats reflects both the initial quality loss accompanying thawing and the higher Hx levels ultimately reached at the borderline level of acceptability. Further study is necessary to appraise fully the potentiality of Hx content as a quality index in thawed scallop meats.

Of considerable technological significance is the fact that, under certain conditions, the hexose monophosphates are present in scallop muscle in amounts sufficient for Maillard browning reactions to occur upon heating. For example, G_6P may produce marked browning in fish flesh under experimental conditions (Tarr 1950). In the past, taste panelists in our laboratory assessing cooked scallop meats have noted (spontaneously) the occurrence of "burned" odors and flavors, and brownish-yellow discolorations in meats stored (unfrozen) for a week or longer before cooking. The present work, in which such effects were also observed, shows that hexose monophosphate at this time would have reached concentrations of 5 μmole/g or greater.

The fact that large amounts of Hx were produced on, and subsequent to, thawing of the prerigor frozen muscle, which initially contained predominantly ATP, indicates that the rate-limiting enzymes involved in the nucleotide degradative sequence from ATP to Hx were strongly activated by the freeze–thaw process. Of particular interest is the 5′-nucleotidase, AMP phosphohydrolase, which in unfrozen scallop muscle appears to be rate-limiting for Hx production (Hiltz and Dyer 1970). This is confirmed in the present work, since thin-layer chromatographic examination (TLC) of extracts of unfrozen muscle revealed considerable AMP still unmetabolized after 13–15 days storage, at Hx levels of 2–3 μmole/g (see Fig. 3c, f). In the thawed meats, both pre- and postrigor, however, TLC of extracts showed that AMP was progressively and completely catabolized during subsequent storage, indicating enhanced AMP-phosphohydrolase activity; at the end of the storage period Hx was the only purine compound detectable on the thin-layer chromatograms. The activation by the freeze–thaw cycle

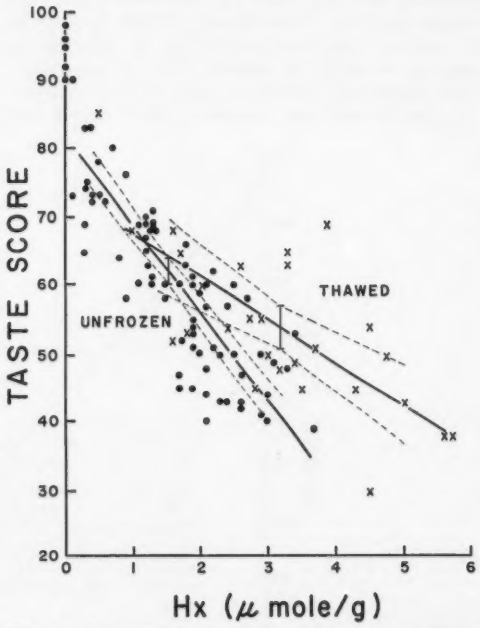

FIG. 5. Regression lines of taste scores on Hx contents in several lots of unfrozen scallop meats stored at 0 or 5 C, and in thawed meats stored at 5 C. 95% confidence limits are shown; $Y = 82–13\ X$ ($n = 82$) and $Y = 74–6.3\ X$ ($n = 27$) for the unfrozen and thawed meats, respectively, where Y = taste score and X = Hx content (μmole/g).

More significantly, we found the browning effect to be particularly apparent in thawed meats which had been frozen prerigor; the increase in intensity of the burned, scorched flavors and odors, and of the pigment formation, observed after 1–2 days storage of the thawed meats, undoubtedly reflects the large increase in hexose monophosphate concentrations to exceptionally high levels (> 20 μmole/g, or 0.5–0.6% wet wt) at that time. No browning was observed in prerigor meats cooked from the frozen state, in agreement with the low G_6P levels.

In general, our findings of extensive biochemical changes with accompanying organoleptic deterioration in top quality scallop meats as a result of thawing and subsequent storage emphasize the inadvisability of thawing meats for retail sale. Moreover, they point out to the consumer the desirability of cooking scallop meats from the frozen state if the excellent flavor and textural qualities of the prerigor quick-frozen muscle are to be maintained. Prerigor freezing is possible under present conditions of commercial handling and processing because the slow overall catabolic rate in scallop muscle may delay rigor up to 4–6 days. Changes on thawing of post-rigor-frozen meats were less extensive.

Acknowledgments

We thank Messrs C. G. Murphy and F. Matthews of the Fisheries Service in Digby, N.S., and Pictou, N.S., respectively, for their cooperation in obtaining live scallops; Mr R. A. Keith for valuable technical assistance in certain phases of the work; and Mr D. E. Turner for excellent care and maintenance of the aquaria.

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Balanced Versus Geostrophic Wind-Stress for Hudson Bay

T. S. MURTY AND K. B. YUEN

Marine Sciences Directorate
Department of the Environment, Ottawa, Ont.

MURTY, T. S., AND K. B. YUEN. 1973. Balanced versus geostrophic wind-stress for Hudson Bay. J. Fish. Res. Board Can. 30: 53–62.

Wind-stresses for Hudson Bay for May and September were computed by the geostrophic relation and the balance equation, making use of data averaged over 30 years. The balanced wind-stress for either strong (September) or weak (May) pressure gradient situations was found to be substantially different from the corresponding geostrophic stress. The wind-generated circulations in the water in Hudson Bay calculated using the balanced and the geostrophic wind-stress showed significant differences, the former agreeing better with the observed average circulation.

MURTY, T. S., AND K. B. YUEN. 1973. Balanced versus geostrophic wind-stress for Hudson Bay. J. Fish. Res. Board Can. 30: 53–62.

Dans la présente étude, la poussée du vent de la baie d'Hudson a été déterminée en mai et septembre à l'aide de l'équation d'équilibre, plus raffinée, basée sur des moyennes de trente ans. La poussée du vent équilibrée avec un gradient prononcé (septembre) ou faible (mai) de pression atmosphérique est très différente du stress géostrophique correspondant. La circulation produite par le vent diffère de façon significative si on la calcule à partir du stress équilibré ou du stress géostrophique, la circulation obtenue par la première méthode s'accordant mieux avec la circulation observée.

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IN circumstances where extensive and representative values of wind-stress are not available for the calcula tions of wind-driven motions, the traditional practice in oceanography has been to use the geostrophic relation. From the pressure field, the geostrophic wind field can be computed using the following equations:

$$U_g = -\frac{1}{\rho_a \cdot f} \cdot \frac{\partial P_a}{\partial y} \tag{1}$$

$$V_g = \frac{1}{\rho_a \cdot f} \cdot \frac{\partial P_a}{\partial x} \tag{2}$$

where U_g and V_g are the x and y components of the geostrophic wind field, ρ_a is the density of the air, f is the coriolis parameter, and P_a is the atmospheric pressure. The x and y components, U_s and V_s, of the surface wind field are determined approximately from the geostrophic winds by a rotation of the geostrophic wind vector through an angle θ and a reduction in magnitude by the ratio r.

$$U_s = r\,(U_g \cdot \cos\theta + V_g \cdot \sin\theta) \tag{3}$$

$$V_s = r\,(-U_g \cdot \sin\theta + V_g \cdot \cos\theta) \tag{4}$$

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The surface wind-stress is estimated from the surface wind by means of an empirical relation. The frictional stress problem is very complex and is not clearly understood at present. It is convenient in the calculations to assume a quadratic stress, given by

$$\tau_s = \rho_a \cdot C_D |V_s| V_s \tag{5}$$

where

$$|V_s| = \sqrt{U_s^2 + V_s^2} = \frac{r}{\rho_a \cdot f}\sqrt{\left(\frac{\partial P_a}{\partial x}\right)^2 + \left(\frac{\partial P_a}{\partial y}\right)^2} \tag{6}$$

Here C_D is the drag coefficient of air over the water surface.

The wind-stress calculated in this manner suffers from the following drawbacks: r and θ are empirical. Moreover, since variation of f is permitted, geostrophic winds develop spurious divergence (Shuman 1957) although this might not be important in computing surface stresses. In addition, the convective acceleration in the primitive momentum equations is completely disregarded.

The balanced winds, in theory, represent a higher order approximation to the true winds than the

geostrophic. There is no spurious divergence associated with the balanced winds. Further, they take into account, though only partly, the convective acceleration terms. In spite of these advantages, the calculation of the wind-stress through the balance equation does not seem to have been made use of in either oceanography or limnology. It should be stressed, however, that the calculation of surface wind-stress through the balance equation is not devoid of empiricism either, especially in extrapolating the higher level balanced winds to the surface.

Our aim in this study was to determine the differences in wind-stress calculated through the use of the balance equation (Petterssen 1953) and the geostrophic relation and then estimate how much different the resultant circulations would be in a water body. We used a simple steady state oceanic model first developed by Rao and Murty (1970) in connection with Great Lakes studies. This model was slightly modified (to include β) to be applicable to Hudson Bay. The fact that the months of May and September (which were selected for this study) are typical, respectively, of spring and fall can be seen from descriptions of the meteorological situation over the Bay by Barber (1967) and Thomson (1968).

Solution of the Balance Equation

In the appendix, the balance equation shown below is derived.

$$-g\nabla^2 Z - \frac{f^2}{2} + V\frac{\partial f}{\partial x} - U\frac{\partial f}{\partial y} - \frac{1}{2}\left(\frac{\partial V}{\partial x} + \frac{\partial U}{\partial y}\right)^2 - 2\left(\frac{\partial U}{\partial x}\right)^2 + \frac{1}{2}\left(f + \frac{\partial V}{\partial x} - \frac{\partial U}{\partial y}\right)^2 = 0 \quad (7)$$

It is also shown in the appendix that the balance equation is a more general relation than the geostrophic relation between the pressure and the wind fields, the nondivergent wind field being represented by a stream-function, ψ, and the pressure field being represented by gZ where Z is the dynamic height of a pressure surface. Charney (1955) showed that this is a mixed hyperbolic-elliptic type of equation. Shuman (1957) showed that this becomes completely elliptic only if

$$g\triangleleft^2 Z + \frac{f^2}{2} + V\frac{\partial f}{\partial x} - U\frac{\partial f}{\partial y} = 0. \quad (8)$$

In practice, for water bodies smaller than the world oceans, the last two terms in (8) can be omitted. In this section we solve the balance equation expressed as a diagnostic relation between the height field, Z, and the balanced wind field defined through the stream-function ψ, in the absence of horizontal divergence. The ellipticity condition given by (8) is almost always satisfied for cyclonic relative vorticity but has to be tested for anticyclonic vorticity.

The first term in equation (8) becomes $\nabla\cdot\frac{1}{\rho}\nabla P$ if one uses a x,y,z,t system. At $Z = 0$, ie. at sea level, ρ and P may be replaced by ρ_0 and P_0 and a balance surface wind can be calculated. However, the balance approximation rests on an uneasy ground when one makes use of the surface pressure field. This is so because of the increased influence of friction. Secondly, when we use surface balance winds in the wind-stress calculation, the usual drag coefficient has to be replaced by a "balanced drag coefficient" that is unknown. In view of these two difficulties we used balanced winds at a higher level (700 mb) in the surface stress calculation.

Nevertheless, we computed the surface stress using the sea level charts and balance wind approximation which we call the modified balance equation. There has already been a precedent in the literature for using the balance winds at the 700-mb level for calculating the surface stress in preference to the geostrophic winds computed from the sea level pressure field (e.g. Cressman 1960; Rao 1969). Following Hubert (1955), Rao (1969) approximated the surface winds on a fine grid at 80% of the winds at the 700-mb level and, on a course grid, at 40% of the 700-mb winds.

Interpolation of isobaric heights at intervals of 200 km or more is satisfactory for the lower tropospheric maps published customarily. If either a finer grid has to be used or some details are needed for specific purposes, these analyses have to be redrawn. This is because operationally it is not always possible to bestow adequate attention to the subsynoptic features. Since in the present study it was intended to interpolate at intervals of 76.2 km, the conventional maps were not useful. As a first approximation we constructed the 700-mb heights using the surface pressure and temperature fields, and the lapse rate between the surface temperature and the temperature at 700 mb. The procedure for solving the balance equation and then computing the wind-stress was broken down into the following steps for a rectangular grid over Hudson Bay:

(1) From the surface weather map, using grid points spaced along the contours, the pressure field over the whole grid was obtained by using a two-dimensional Spline interpolation and then six Laplacian smoothings were used to reduce the noise.

(2) Similar analyses were performed on the temperature fields at the surface and the 700-mb level.

(3) The average of $T_{surface}$ and T_{700mb} was calculated at every grid point and is represented by T_r.

(4) The virtual temperature was calculated as follows. From the table titled "virtual temperature increase of saturated air" in the Smithsonian meteorological tables, the increment of temperature corresponding to T_r for 850 mb was obtained. Here 850 mb is an average value of the pressure between sea level and the 700-mb level. This increment was added to T_r to give the virtual temperature in degrees Celsius. This was converted into degrees Kelvin by adding 273 and is denoted by T_V.

(5) The dynamic height, Z, was calculated for all the grid points using the following formula.

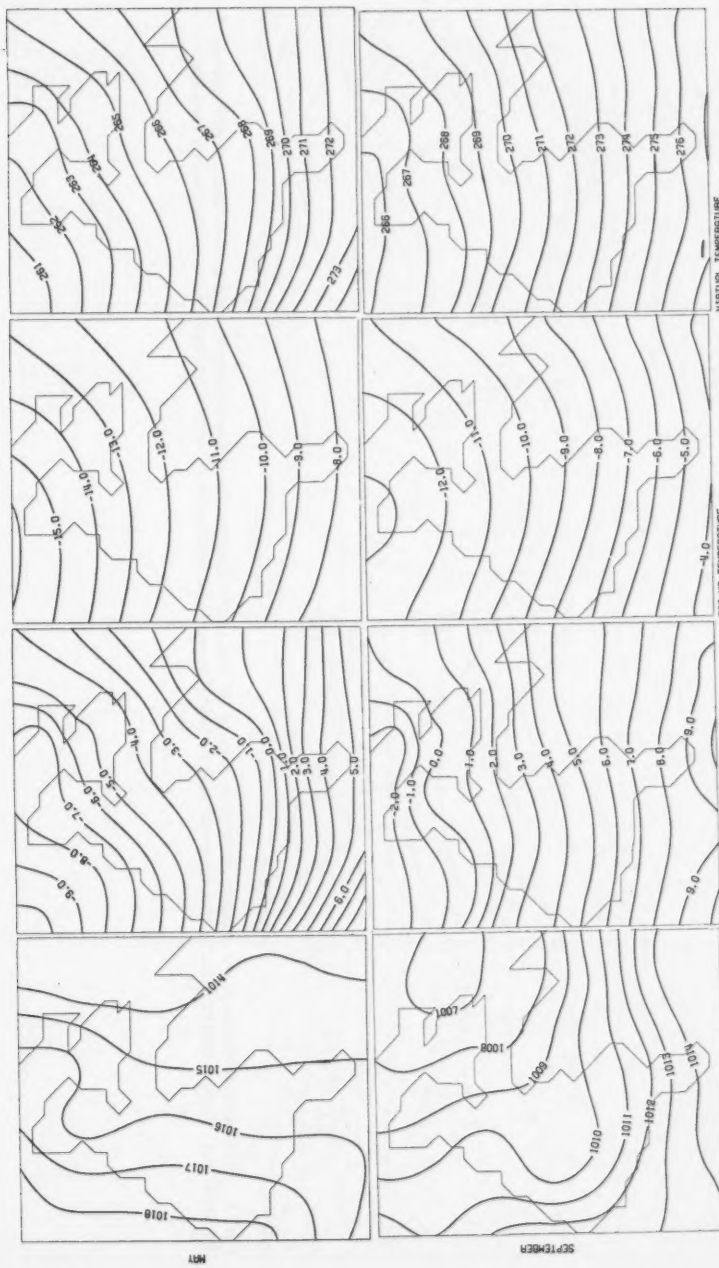

FIG. 1. Sea level pressure (mb), surface temperature (C), temperature at 700 mb (C), and mean virtual temperature at 850 mb (K) for Hudson Bay for May (*top*) and September (*bottom*). Surface data are averaged over 30 years and the upper air data over 10 years (Meteorological Branch 1967 and Titus 1967).

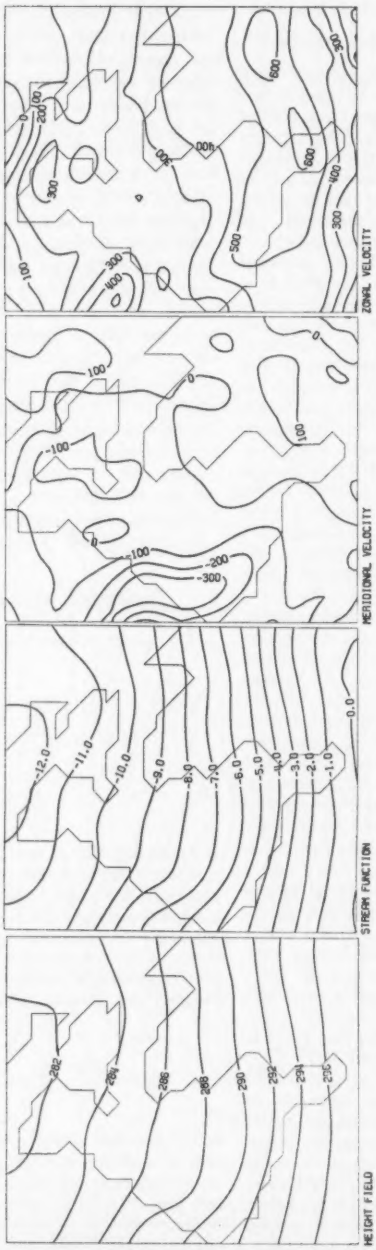

FIG. 2. Geopotential heights of the pressure surfaces in units of 10^3 cm, stream-function for the nondivergent wind field in units of 10^{10} cm^2·sec^{-1}, the meridional velocity field (cm·sec^{-1}), and the zonal velocity field (cm·sec^{-1}) for September computed through the use of the balance equation.

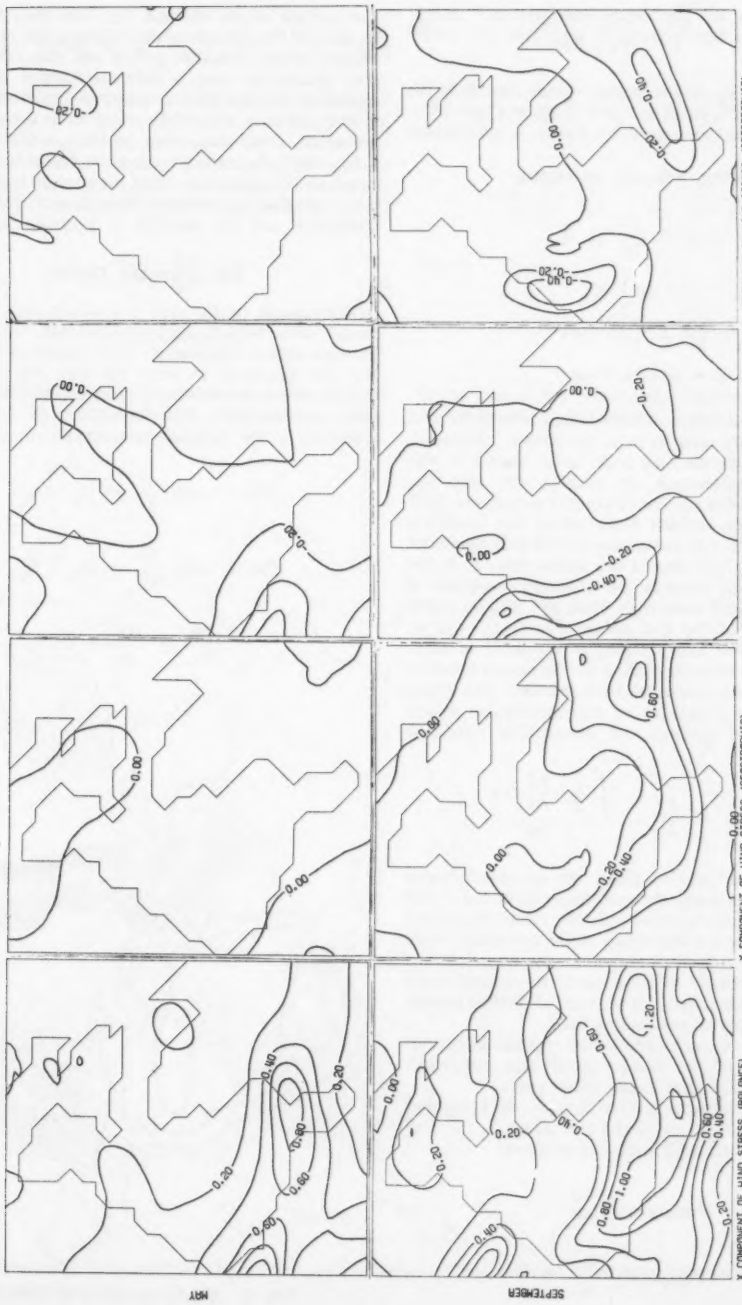
X COMPONENT OF WIND STRESS (BALANCE)
X COMPONENT OF WIND STRESS (GEOSTROPHIC)
Y COMPONENT OF WIND STRESS (BALANCE)
Y COMPONENT OF WIND STRESS (GEOSTROPHIC)
SEPTEMBER
MAY

FIG. 3. Wind-stress components for May (*top*) and September (*bottom*) in units of dynes·cm^{-2}.

$$Z = \frac{2R}{g} \frac{T_V(P_b - 700)}{(P_b + 700)} \qquad (9)$$

where P_b is the sea level pressure in mb. Here R is the gas constant and is equal to 2.870×10^6 ergs·g^{-1}·°K^{-1}. The units of Z in (9) are cm if the gravity, g, is expressed in cm·sec^{-2}.

(6) The ellipticity test was performed.

$$\nabla^2 Z + \frac{f^2}{2g} > 0$$

$$f = f_0 + \beta y$$

$$f_0 = 1.1 \times 10^{-4} \text{ sec}^{-1}$$

$$\beta = 10^{-11} \text{ m}^{-1}\cdot\text{sec}^{-1}$$

Here β is the variation of the coriolis parameter with latitude, generally referred to as the Rossby parameter. In the calculations the map scale factor should be also taken into consideration. In general, the ellipticity condition is satisfied at most of the grid points, but there might exist some isolated areas where this condition might be violated. For these cases we followed Shuman's (1957) procedure and altered the height field, Z, in the following manner. Suppose the ellipticity condition is violated at the grid point (i, j). Then $\frac{1}{4}(\nabla^2 Z)_{i,j}$ is added to $\nabla^2 Z$ values at the grid points $(i-1, j)$, $(i+1, j)$, $(i, j+1)$, and $(i, j-1)$ and $(\nabla^2 Z)_{i,j}$ is set equal to zero.

(7) Next the boundary values on the stream-function entering the balance equation were specified. The values of ψ along the boundary of the rectangular region were determined through the geostrophic condition

$$\frac{\partial \psi}{\partial S} = \frac{g}{f} \cdot \frac{\partial Z}{\partial S} - \frac{\oint \frac{g}{f} \cdot \frac{\partial Z}{\partial S} \, dS}{\int dS}$$

where S is taken positive along the counterclockwise direction. In this study the grid intervals in the x and y directions are both taken as $\triangle S$. The values of $\triangle Z/\triangle S$ were obtained from a knowledge of the boundary height field. This geostrophic condition at the boundaries is somewhat inconsistent with the use of the balance equation at the interior points, but for want of a better boundary condition this approach was used.

(8) Using the Gaussian elimination method the stream-function, ψ, at all the interior points was calculated. The values of gZ/f serve as the initial guess.

(9) From the stream-function field, ψ, the zonal and meridional wind fields at all the grid points at the 700-*mb* level were calculated using the relations

$$U_{700mb} = -\frac{\partial \psi}{\partial y}$$

$$V_{700mb} = \frac{\partial \psi}{\partial x}$$

The surface winds, U_S and V_S, were then taken as a fraction of the 700-mb winds following the precedent of Hubert (1955), Cressman (1960), and Rao (1969). However, instead of using a different fraction in different regions as Hubert did (he varied the fraction from 0.4 to 0.8), we took the surface winds to be 0.6 of the 700-mb winds. From this, using the drag coefficient $C_D = 2.6 \times 10^{-3}$, the surface wind-stress was calculated from equation (5). Although using a constant value for C_D is not satisfactory, accurate observational data on drag coefficients are not available at present.

The Oceanic Model

The oceanic model used is a steady state linearized model with bottom topography taken into account through vertical integration of the equations of motion. We are interested in May (spring) and September (fall), and the assumption of homogeneity is a reasonable approximation. The equations of motion and continuity in the vertically integrated form are

$$-fN = -gH \frac{\partial \eta}{\partial x} + \tau_{s_x} - \tau_{b_x} \qquad (10)$$

$$fM = -gH \frac{\partial \eta}{\partial y} + \tau_{s_y} - \tau_{b_y} \qquad (11)$$

$$\frac{\partial M}{\partial x} + \frac{\partial N}{\partial y} = 0 \qquad (12)$$

FIG. 4. Depth contours in meters.

where

$$(M,N) \equiv \int_{-H}^{\eta} (U,V)\, dZ \tag{13}$$

Here M and N are the x and y components of the volume transport; τ_{s_x} and τ_{s_y} are the x and y components of the surface stress, and τ_{b_x} and τ_{b_y} are the x and y components of the bottom stress. To be consistent with the linearization, the upper limit of integration in (13) is taken as zero instead of η. Following Stommel (1948) we assume that

$$\begin{aligned} \tau_{b_x} &= RM \\ \tau_{b_y} &= RN \end{aligned} \tag{14}$$

where $R \equiv \dfrac{K}{H}$, K being a constant.

This formulation for bottom stress recognizes that the effects are felt more in shallow areas than in deeper areas. The next step is to form the vorticity equation from the equations of motion and continuity:

$$R\left(\frac{\partial N}{\partial x} - \frac{\partial M}{\partial y}\right) + \left(N\,\frac{\partial R}{\partial x} - M\,\frac{\partial R}{\partial y}\right) + \beta N + g\left(\frac{\partial H}{\partial x}\cdot\frac{\partial \eta}{\partial y} - \frac{\partial H}{\partial y}\cdot\frac{\partial \eta}{\partial x}\right) - \tau = 0 \tag{15}$$

where

$$\tau \equiv \frac{\partial \tau_{s_y}}{\partial x} - \frac{\partial \tau_{s_x}}{\partial y} \tag{16}$$

is the curl of the wind-stress. Define vorticity, ξ, and stream-function, ψ, through

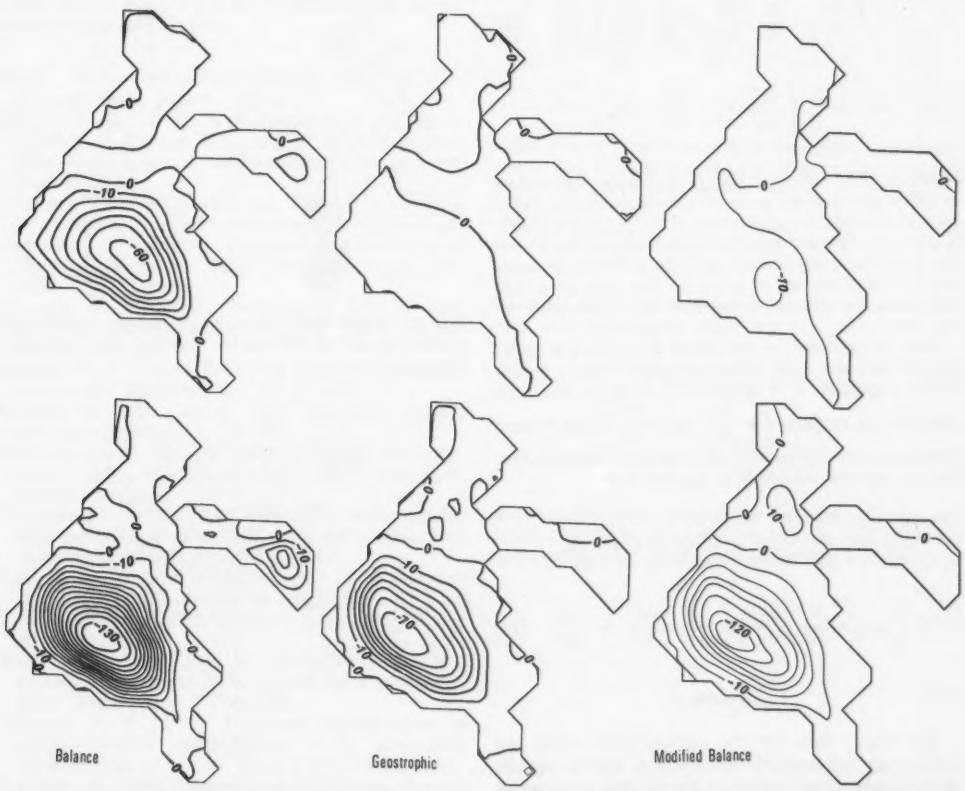

FIG. 5. Vertically integrated stream-function field in units of 10^{12} cm^3·sec^{-1} for water circulation for May (*top*) and September (*bottom*).

$$\xi \equiv \frac{\partial N}{\partial x} - \frac{\partial M}{\partial y} = \nabla^2\psi \qquad (17)$$

and

$$M \equiv -\frac{\partial \psi}{\partial y} \qquad (18)$$

$$N \equiv \frac{\partial \psi}{\partial x} \qquad (19)$$

In (16), elimination of $\frac{\partial \eta}{\partial x}$ and $\frac{\partial \eta}{\partial y}$ through (10) and (11) gives

$$R\nabla^2\psi + \left(\frac{\partial R}{\partial x} - \frac{R}{H}\frac{\partial H}{\partial x} - \frac{f}{H}\frac{\partial H}{\partial y} + \beta\right)\frac{\partial \psi}{\partial x} + \left(\frac{\partial R}{\partial y} - \frac{R}{H}\frac{\partial H}{\partial y} + \frac{f}{H}\frac{\partial H}{\partial x}\right)\frac{\partial \psi}{\partial y} \qquad (20)$$
$$= \tau - \frac{1}{H}\left(\frac{\partial H}{\partial x}\cdot\tau_{sy} - \frac{\partial H}{\partial y}\cdot\tau_{sx}\right)$$

Equation (20), which is elliptic in nature, was solved at all the grid intervals using the Gauss elimination method and through the application of the boundary condition $\psi = 0$. In this simple model we neglected the islands and took into consideration only the coastline. In equation (20) the last set of terms on the right-hand side represents the interaction between the components of the wind-stress and the depth gradients.

After computation of the stream-function, the deviation of the water level, η, was computed from a double Taylor expansion of η in terms of x and y, using the following expressions for $\frac{\partial \eta}{\partial x}$ and $\frac{\partial \eta}{\partial y}$. It can be seen from equations (10) and (11) that the static level gradient due to pressure difference is ignored here.

$$\frac{\partial \eta}{\partial x} = \frac{f}{gH}\frac{\partial \psi}{\partial x} + \frac{R}{gH}\frac{\partial \psi}{\partial y} + \frac{\tau_{sy}}{gH}$$

$$\frac{\partial \eta}{\partial y} = -\frac{R}{gH}\frac{\partial \psi}{\partial x} + \frac{f}{gH}\frac{\partial \psi}{\partial y} + \frac{\tau_{sy}}{gH} \qquad (21)$$

Results

The input data for the computation using the balance equation are shown in Fig. 1, and the results of the intermediate steps in Fig. 2. The wind-stress components (Fig. 3) reveal that for May the geostrophic wind-stress, which is smaller in magnitude than the balanced wind-stress, does not give an accurate picture over most of Hudson Bay. This could be inferred by comparing the observed circulation with those determined from balanced and geostrophic wind-stresses (Fig. 5, 7). For September the geostrophic wind-stress agrees more closely with the

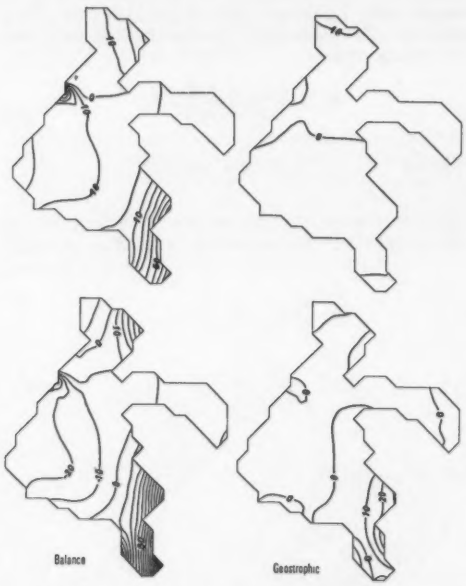

FIG. 6. Water level deviations from the equilibrium position in units of 10^{-2} cm for May (*top*) and September (*bottom*).

FIG. 7. Observed average circulation in Hudson Bay (Anon. 1959, Fig. 5, p. 105).

balanced wind-stress and, thus, we infer that when the pressure gradient is strong over Hudson Bay the geostrophic wind-stress is a reasonable approximation for the real wind-stress.

In Fig. 4 we show the depth contours of Hudson Bay and in Fig. 5 a comparison among the circulations determined from three types of wind-stresses: balanced, geostrophic, and modified-balanced. It can be seen that the modified-balanced approximation is not a particularly significant improvement over the geostrophic approximation. The water level deviations from the equilibrium position computed with balanced and geostrophic wind-stresses (Fig. 6) are consistent with the earlier deduction that geostrophic wind-stress is a poor approximation for May but is reasonable for September.

Acknowledgments

The authors thank Mr J. D. Taylor for programming the numerical calculations.

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Appendix

DERIVATION OF THE BALANCE EQUATION

The primitive horizontal equations of motion in a x,y,P,t coordinate system under the hydrostatic assumption for the pressure field are given by

$$\frac{\partial U}{\partial t} + U\frac{\partial U}{\partial x} + V\frac{\partial U}{\partial y} + \omega\frac{\partial U}{\partial P} = -g\frac{\partial Z}{\partial x} + fV \tag{A.1}$$

$$\frac{\partial V}{\partial t} + U\frac{\partial V}{\partial x} + V\frac{\partial V}{\partial y} + \omega\frac{\partial V}{\partial P} = -g\frac{\partial Z}{\partial y} - fU \tag{A.2}$$

where ω is the vertical velocity in the isobaric coordinate system and Z is the geopotential height of the pressure surface. The continuity equation is

$$\frac{\partial U}{\partial x} + \frac{\partial V}{\partial y} + \frac{\partial \omega}{\partial P} = 0 \tag{A.3}$$

Invoking the quasi-geostrophic approximation, (A.1) and (A.2) reduce to

$$0 = -g\frac{\partial Z}{\partial x} + fV \tag{A.4}$$

$$0 = -g\frac{\partial Z}{\partial y} - fU \tag{A.5}$$

This approximation is known as the geostrophic approximation in the x,y,z system. From (A.3) and (A.4) the quasi-geostrophic wind components can be written as

$$U_g = -\frac{g}{f}\cdot\frac{\partial Z}{\partial y} \tag{A.6}$$

$$V_g = \frac{g}{f}\cdot\frac{\partial Z}{\partial x} \tag{A.7}$$

Elsasser (1968) ignored the variation of the coriolis parameter f with latitude and replaced f with f_o in (A.6) and (A.7) and called these two equations together the "poor man's balance equation."

To derive the balance equation we take the divergence of the horizontal momentum equations. Equations (A.1) and (A.2) can be cross-differentiated with respect to x,y and added and then the use of the continuity equation (A.3) gives

$$\frac{\partial D}{\partial t} + U\frac{\partial D}{\partial x} + V\frac{\partial D}{\partial y} + \omega\frac{\partial D}{\partial P} + \left(\frac{\partial U}{\partial x}\right)^2 + \frac{\partial V}{\partial x}\cdot\frac{\partial U}{\partial y} + \frac{\partial \omega}{\partial x}\cdot\frac{\partial U}{\partial P} + \frac{\partial U}{\partial y}\cdot\frac{\partial V}{\partial x} + \left(\frac{\partial V}{\partial y}\right)^2 + \frac{\partial \omega}{\partial x}\cdot\frac{\partial V}{\partial P} = -g\nabla^2 Z + f\left(\frac{\partial V}{\partial x} - \frac{\partial U}{\partial y}\right) + V\frac{\partial f}{\partial x} - U\frac{\partial f}{\partial y} \quad (A.8)$$

where

$$D \equiv \frac{\partial U}{\partial x} + \frac{\partial V}{\partial y} \quad (A.9)$$

is the divergence of the horizontal wind field.

According to the Helmholtz principle, any arbitrary velocity field $\mathbb{V}$ can be represented as the sum of a rotational (nondivergent) velocity $\mathbb{V}_\psi$ and an irrotational (divergent) velocity $\mathbb{V}_\phi$

$$\mathbb{V} \equiv \mathbb{V}_\psi + \mathbb{V}_\phi \quad (A.10)$$

Let U_r and V_r be the x and y components of the irrotational part of the motion and U_d and V_d be the corresponding components for the nondivergent part. Then we have the following relations:

$$U_r = -\frac{\partial \psi}{\partial y} \qquad U_d = -\frac{\partial \phi}{\partial x}$$

$$V_r = \frac{\partial \psi}{\partial x} \qquad V_d = -\frac{\partial \phi}{\partial y}$$

$$\frac{\partial U_r}{\partial x} + \frac{\partial V_r}{\partial y} = 0$$

$$\frac{\partial U_d}{\partial y} - \frac{\partial V_d}{\partial x} = 0 \quad (A.11)$$

Also we have

$$D = \frac{\partial U_d}{\partial x} + \frac{\partial V_d}{\partial y} = -\nabla^2\phi \quad (A.12)$$

and

$$\omega = \int D\cdot\partial P \quad (A.13)$$

In large scale atmospheric systems (> 500 km scale), the divergence $\frac{\partial U}{\partial x} + \frac{\partial V}{\partial y}$ is small compared with the vorticity $\frac{\partial V}{\partial x} - \frac{\partial U}{\partial y}$. Hence, ($U_r$, V_r) are about an order of magnitude larger than (U_d, V_d). Since the vertical velocity ω is determined completely from (U_d, V_d) as can be seen from (A.13), the terms in (A.8) that contain D or ω are smaller than those that do not contain them. Hence, following Lorenz (1960), we write (A.8) as

$$2\left(\frac{\partial U_r}{\partial x}\right)^2 + 2\frac{\partial V_r}{\partial x}\cdot\frac{\partial U_r}{\partial y} = -g\nabla^2 Z + f\left(\frac{\partial V_r}{\partial x} - \frac{\partial U_r}{\partial y}\right) + V_r\frac{\partial f}{\partial x} - U_r\frac{\partial f}{\partial y} \quad (A.14)$$

Since we are dealing exclusively with U_r and V_r, it is convenient to drop the subscript r. Equation (A.14) may be rearranged as

$$-g\nabla^2 Z - \frac{f^2}{2} + V\frac{\partial f}{\partial x} - U\frac{\partial f}{\partial y} - \frac{1}{2}\left(\frac{\partial V}{\partial x} + \frac{\partial U}{\partial y}\right)^2 - 2\left(\frac{\partial U}{\partial x}\right)^2 + \frac{1}{2}\left(f + \frac{\partial V}{\partial x} - \frac{\partial U}{\partial y}\right)^2 = 0 \quad (A.15)$$

This is the so-called balance equation.

Thiobarbituric Acid Value, Total Long-Chain Free Fatty Acids, and Flavor of Pacific Halibut (*Hippoglossus stenolepis*) and Chinook Salmon (*Oncorhynchus tshawytscha*) Frozen at Sea[1]

J. R. BOTTA,[2] J. F. RICHARDS

Department of Food Science
University of British Columbia, Vancouver, B.C.

AND N. TOMLINSON

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver 8, B.C.

BOTTA, J. R., J. F. RICHARDS, AND N. TOMLINSON. 1973. Thiobarbituric acid value, total long-chain free fatty acids, and flavor of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) frozen at sea. J. Fish. Res. Board Can. 30: 63–69.

The effects of brine-freezing and plate-freezing at sea upon 2-thiobarbituric acid (TBA) values, concentration of long-chain free fatty acids (FFA), and flavor of Pacific halibut and chinook salmon were determined at intervals during subsequent storage of the glazed, polyethylene covered fish at –30 C. Evaluations were conducted on both outside and inside muscle.

Brine-frozen samples of outside muscle generally had higher TBA values than plate-frozen samples and the differences were most pronounced at intermediate storage times (26–45 weeks).

Method of freezing significantly ($P \leq 0.05$) affected the concentration of total FFA in Pacific halibut, and the total FFA concentration of both Pacific halibut and chinook salmon significantly increased with length of frozen storage. For both species the concentration of total FFA was significantly greater in outside than in inside muscle.

Significant flavor differences between brine- and plate-frozen samples of outside muscle were evident at intermediate storage times for both species. For inside muscle of halibut, the significance of flavor differences generally increased with storage time whereas flavor differences for inside muscle of salmon were consistently nonsignificant.

Taste panel results and TBA values indicated that, in comparison to plate-freezing, brine-freezing impaired the quality of outside muscle of Pacific halibut and chinook salmon during the early stages of frozen storage.

BOTTA, J. R., J. F. RICHARDS, AND N. TOMLINSON. 1973. Thiobarbituric acid value, total long-chain free fatty acids, and flavor of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) frozen at sea. J. Fish. Res. Board Can. 30: 63-69.

Les auteurs ont déterminé les effets de la congélation par la saumure et sur plaques en mer sur la teneur en acide 2-thiobarbiturique (TBA), la teneur en acides gras libres à longue chaîne (FFA) et la saveur du flétan du Pacifique et du saumon chinook. Ces mesures ont été effectuées périodiquement au cours de l'entreposage à –30 C du poisson givré et recouvert de polyéthylène. Elles ont été faites à la fois sur le muscle externe et sur le muscle interne.

En général, les échantillons de muscle externe congelés en saumure ont des valeurs de TBA plus élevées que ceux congelés sur plaques. Les différences sont plus marquées à une durée intermédaire d'entreposage (26–45 semaines).

La méthode de congélation affecte de façon significative ($P \leq 0.05$) la teneur en FFA total du flétan. La teneur en FFA total augmente de façon significative chez le flétan aussi

[1]This paper is the first in a series of three on the effects of brine-freezing and plate-freezing at sea on the quality of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*).

[2]Present address: Department of Food Science, Louisiana State University, Baton Rouge, La. 70803, USA.

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bien que chez le saumon avec la durée de l'entreposage réfrigéré. Chez les deux espèces, la teneur en FFA total est nettement plus élevée dans le muscle externe que dans le muscle interne.

Il existe chez les deux espèces des différences significatives dans la saveur d'échantillons de muscle externe congelés en saumure ou sur plaques, à des durées intermédiaires d'entreposage. Dans le muscle interne du flétan, les différences de saveur s'accentuent ordinairement avec la durée d'entreposage, alors que pour le muscle interne, ces différences de saveur sont uniformément non significatives.

Les essais de dégustation et les valeurs de TBA démontrent que la congélation par la saumure, comparativement à la congélation sur plaques, abaisse la qualité du muscle externe du flétan du Pacifique et du saumon chinook durant les premiers stades d'entreposage refrigéré.

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THERE is currently increased interest in the freezing of fish at sea in Canada's Pacific coast fishery. Advantages of freezing fish at sea, as compared to preserving them with flake ice or refrigerated sea water (RSW), are: extension of fishing to more distant grounds, landing fish of higher and more uniform quality, landing of capacity loads, and leveling out supplies of raw material for the processing plant (Oldershaw 1955; Eddie 1959). However, the method used to freeze fish at sea may affect the quality of the frozen-stored product (Harrison and Roach 1953; Kuprianoff 1956).

Although it is not uncommon for the flesh of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) to be kept in frozen storage for extended periods of time before marketing, the effect of freezing method on the quality of Pacific species has not been extensively studied.

The purpose of the present study was to determine the effects of brine- and plate-freezing at sea and length of subsequent frozen storage upon 2-thiobarbituric acid (TBA) values, flesh content of long-chain free fatty acids (FFA), and flavor of Pacific halibut and chinook salmon. Subsequent papers will deal with flesh *p*H and color, thaw drip, mineral concentration, and individual free fatty acids.

Materials and Methods

Catching and Freezing the Fish

Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) were caught by longline on June 7, 1969 and by seine on July 21, 1969, respectively, in waters adjacent to the northern end of Vancouver Island. Three in-rigor fish of each species were frozen in a vertical plate-freezer at –30 C for 3.5 hr and three others were frozen in a 13% NaCl brine at –5.6 C for 9.5 hr (salmon) and 47 hr (halibut), the times being those necessary to reach temperature equilibration.

After freezing the fish were glazed twice, using an ice-water mixture, enclosed in polyethylene and stored at –30 C until required.

Sampling

Each fish was sampled five times during storage by sawing ½-inch thick steaks from the anterior end of the fish. The remainder was immediately reglazed, bagged, and returned to the freezer. Prior to the first analysis of each species, the six fish were randomly divided into three pairs, each pair consisting of one brine-frozen and one plate-frozen fish. Samples were taken from each pair at each sampling time.

Analysis

All chemical and organoleptic analyses were conducted on both inside and outside muscle, the red lateral muscle being included with the outside sample (Fig. 1).

TBA values were determined by the procedure of Tarladgis et al. (1960) as modified by Castell et al. (1966b). Ten grams of frozen tissue and 1.10 g of the disodium salt of ethylenediaminetetraacetic acid (EDTA) were homogenized with 50 ml distilled water for 90 sec

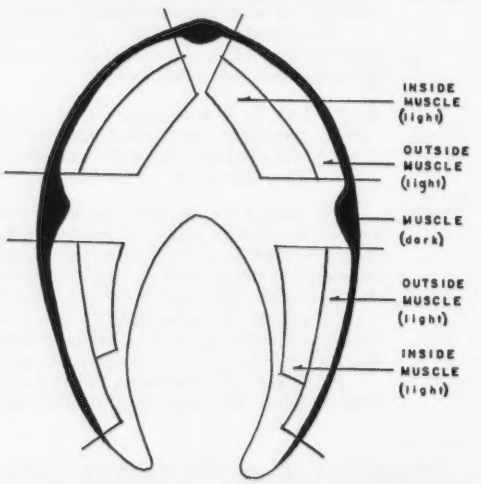

FIG. 1. Cross section of a fish showing location of inside and outside muscle. Outside muscle samples included both light and dark muscle.

in a Serval Omni-Mixer. The final volume of the mixture prior to acidification was 110 ml. The acidified mixture was distilled to give a total of 50 ml of distillate in 18–20 min.

Long-chain FFA were isolated and analyzed as described by Botta et al. (1973a) and are expressed herein as micrograms per gram of flesh.

Differences in flavor between brine- and plate-frozen fish were determined using the triangle test as outlined by Larmond (1967). The samples were prepared for organoleptic evaluation by wrapping the steaks in aluminum foil and steaming for 12 min. The steaks were then unwrapped and divided into samples of inside and outside muscle (Fig. 1). The flavor of the samples was evaluated by a taste panel consisting of five experienced panel members. Red lights were used to mask any color differences among samples. Each panel member was instructed to include the red muscle when tasting outside muscle samples.

The TBA values and long-chain FFA data were statistically analyzed using a split–split plot arrangement of treatments. Pairs were considered fixed in all analyses. The absolute differences in TBA values and FFA concentration between the brine-frozen and the plate-frozen samples were correlated with the taste panel results, expressed as the number of correct identifications.

Results and Discussion

TBA Value

The mean TBA value of brine-frozen Pacific halibut was significantly ($P \leq 0.05$) greater than that of plate-frozen (Table 1, Fig. 2). The difference in TBA value between halibut outside and inside muscle was not consistent during storage. The difference in TBA value in favor of outside muscle was much greater at intermediate storage times than for shorter or longer storage durations (Fig. 2). This effect is reflected by the significant ($P \leq 0.05$) location × time interaction (Table 1).

The significant ($P \leq 0.05$) method × location interaction revealed by analysis of variance of TBA value of chinook salmon (Table 1) is graphically represented in Fig. 3. It is obvious that the extremely high TBA values for outside muscle of brine-frozen salmon at 26 and 40 weeks of storage were largely responsible for the interaction. Length of frozen storage had a highly significant effect ($P \leq 0.01$) on mean TBA value (Table 1). Mean TBA values at the first and last two sampling periods were similar (Table 2). The highest mean TBA value occurred at 26 weeks of storage and was significantly higher ($P \leq 0.05$) than those at 9, 77, and 58 weeks (Table 2).

The TBA values for the inside muscle of both species were relatively similar for both the brine- and plate-frozen samples, suggesting little difference in degree of oxidation in inside muscle frozen by the two different methods (Fig. 2 and 3). In general, with both species, the outside muscles from the brine-frozen samples had higher TBA values than those from corresponding plate-frozen samples, thus indicating greater oxidative rancidity, particularly at 26–31 weeks of frozen storage.

Table 1. Mean squares of TBA values (× 10^3) and total FFA concentrations (expressed as micrograms FFA per gram of flesh × 10^{-3}) of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*). Significance at the 5 and 1% levels indicated by * and **, respectively.

		Mean squares			
		Pacific halibut		Chinook salmon	
Source	df	TBA value	FFA	TBA value	FFA
Pairs	2	11.23	6.31	0.46	46.81
Method[a]	1	20.80*	232.23*	87.55	43.48
Error a	2	0.33	1.30	11.50	749.99*
Location	1	80.89	421.21*	249.36	4522.90*
M × L	1	14.26	100.01	74.20*	12.56
Error b	4	2.82	38.34	4.36	237.38
Time	4	7.56	91.36	41.54	2369.20**
M × T	4	4.51	0.18	20.50	180.36
L × T	4	12.00*	49.39	22.36	351.16
M × L × T	4	3.97	17.44	14.55	191.06
Error c	32	4.42	40.63	9.03	187.41

[a]Methods were tested by error a. Locations and M × L were tested using error b. All other terms were tested using error c.

Awad et al. (1969) observed that the TBA value of freshwater whitefish, stored at −10 C, steadily increased with time of frozen storage. In contrast, Castell et al. (1966a) reported that the lipids of cod muscle stored at −18 C and at −25 C for 270 and 1290 days, respectively, did not undergo oxidation as measured by the TBA method. Castell et al. (1966a) also found that during frozen storage the lipids of cod muscle became markedly more resistant to metal − (Cu^{2+} or Fe^{2+}) or to hemoglobin-catalyzed oxidation. They suggested that the free fatty acids may have reacted with proteins or some other component of the muscle in a manner that protected their double bonds against oxidation. An analogous reaction may have contributed to the decrease in the TBA values that occurred during the present study. Alternatively the decline may have been due to unavailability of malonaldehyde. Buttkus (1967) showed that malonaldehyde reacts with the ε-amino groups of trout myosin. The rate of this reaction

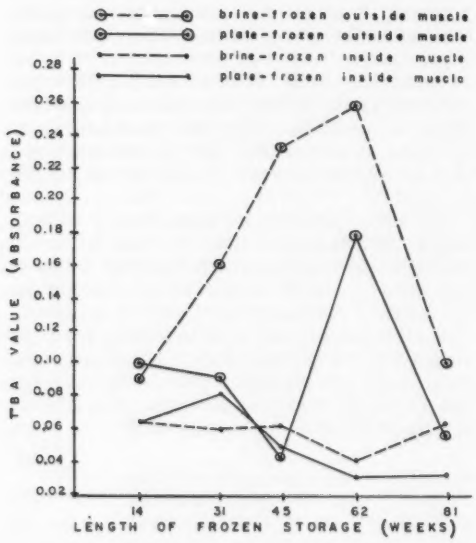

FIG. 2. Average T.B.A. values for Pacific halibut (*Hippoglossus stenolepis*) stored at −30 C.

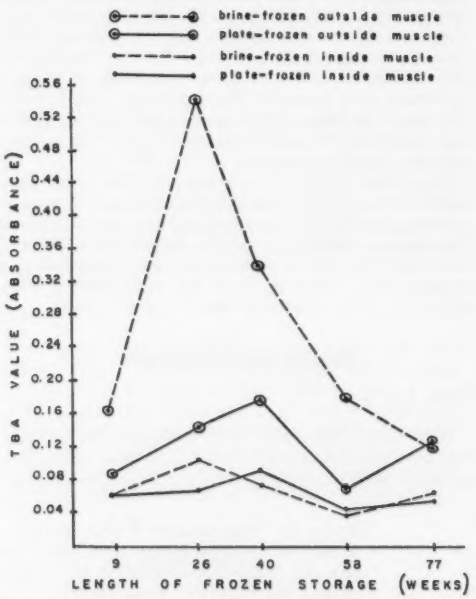

FIG. 3. Average T.B.A. values for chinook salmon (*Oncorhynchus tshawytscha*) stored at −30 C.

TABLE 2. Storage time means and results of Duncan's New Multiple Range Test for total FFA concentration and TBA value by species. Means are presented only where the storage time effect was found significant ($P \leq 0.05$) in the analysis of variance. Means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

Pacific halibut		Chinook salmon		
Weeks of storage	FFA concentration (*μg/g flesh*)	Weeks of storage	FFA concentration (*μg/g flesh*)	TBA value
14	192.0 a	9	501.5 a	0.0929 ac
31	408.5 b	26	969.8 b	0.2138 b
45	397.3 b	40	1583.2 c	0.1695 ab
62	320.0 ab	58	1212.3 bc	0.0820 c
81	357.2 ab	77	1531.6 c	0.0902 ac

was less at 0 C than at +20 C while the reaction rate at −20 C was almost equal to that at +20 C. Buttkus (1969) also showed that malonaldehyde reacted with cysteine and methionine. Kwon et al. (1965) reported that thiobarbituric acid reactive substances react with protein, and in tuna stored at −18 C for 8 months became partially unrecoverable for the TBA test.

An additional factor probably contributing to the decrease in TBA values observed in the present study is the fact that oxygen is necessary for oxidative rancidity to occur (Lundberg 1961) and since the fish were heavily glazed rancidity could probably only continue until the oxygen initially present in the tissue was exhausted.

Total Long-Chain FFA

With halibut the concentration of total FFA was significantly ($P \leq 0.05$) greater in the plate-frozen than in the brine-frozen samples (Table 1), and was significantly ($P \leq 0.05$) greater in the outside than in the inside muscle (Table 3). Although analysis of variance showed that the time effect was nonsignificant ($P \leq 0.085$) Duncan's New Multiple Range test indicated that the concentration of total FFA at 31 weeks of frozen storage was significantly ($P \leq 0.05$) greater than the concentration at 14 weeks but not significantly different from the concentrations at 45, 62, and 81 weeks (Table 2).

The concentration of total FFA was not significantly ($P \leq 0.05$) different for brine- and plate-frozen chinook salmon (Table 1) but there was significantly ($P \leq 0.05$) more total FFA in outside than in inside muscle (Tables 1 and 3). In addition, the concentration of FFA was significantly ($P \leq 0.01$) affected by length of storage (Tables 1 and 2).

In general, with both species, the mean FFA concentration increased during frozen storage with a large increase during the first 40 weeks of storage (Table 2). This rapid increase in total FFA concentration, followed by a much slower increase is similar to the pattern observed in Pacific gray cod and Atlantic cod by Wood and Haqq (1962), Olley et al. (1969), and Anderson and Ravesi (1970).

Table 3. Method and location means for total FFA concentration by species. (ns = no significant difference at the 5% level; * = Significant at the 5% level.)

	Mean FFA conc. ($\mu g/g$ *flesh*)			
	Pacific halibut		*Chinook salmon*	
Brine-frozen	272.∞	*	1132.7	n.s.
Plate-frozen	397.2		1186.6	
Inside muscle	251.2	*	885.1	*
Outside muscle	418.8		1434.2	

Organoleptic Analysis

The difference in flavor between brine- and plate-frozen samples of outside muscle was nonsignificant at the first sampling period. However, for both species the taste panel found that the differences in flavor between outside muscle samples from brine- and plate-frozen fish reached a highly significant ($P \leq 0.01$) maximum at the second analysis period (i.e., at the end of approximately 29 weeks) and then steadily decreased (Fig. 4). This observation may be related to the higher salt content in the brine-frozen outside muscle samples (Botta et al. 1973b) which may have accelerated the induction period of lipid oxidation. In chinook salmon the greatest difference in TBA values between brine- and plate-frozen samples occurred at the second sampling period and in halibut at the third sampling period. It is not inconceivable that after approximately 30 weeks of frozen storage the plate-frozen samples may have begun to 'catch-up' with the brine-frozen samples in development of rancidity and off-flavors.

With halibut inside muscle, significant ($P \leq 0.05$) differences in flavor between brine- and plate-frozen

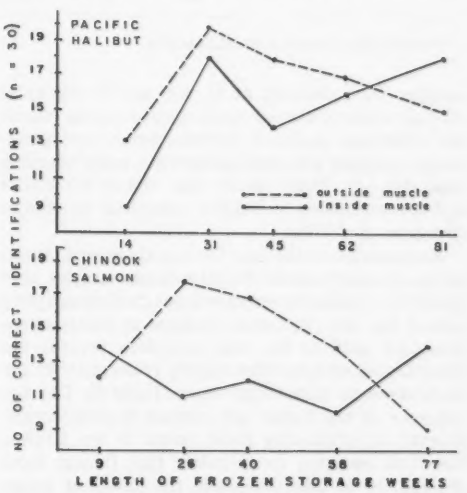

Fig. 4. Ability of panelists to correctly identify the odd sample in triangle tests of plate- and brine-frozen samples of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*).

TABLE 4. Acceptability preferences for Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*).

Location	Length of frozen storage (*weeks*)	Preferences[a] Plate-frozen (%)	Brine-frozen (%)	No. Pref. (%)
		Pacific halibut		
Outside muscle	14	69	31	
	31	60	40	
	45	39	50	11
	62	29	59	12
	81	47	53	
Inside muscle	14	22	78	
	31	50	50	
	45	43	50	7
	62	50	38	13
	81	44	28	28
		Chinook salmon		
Outside muscle	9	67	33	
	26	56	44	
	40	59	35	6
	58	50	50	
	77	33	56	11
Inside muscle	9	57	43	
	26	55	45	
	40	50	50	
	58	–	70	30
	77	50	21	29

[a]Percentage of correct identifications.

samples were detected at 31, 62, and 81 weeks of storage whereas with chinook salmon inside muscle the difference in flavor between brine- and plate-frozen samples was nonsignificant at each sampling time (Fig. 4). These results may reflect the higher salt concentrations in halibut compared to salmon (Botta et al. 1973b).

Examination of the data for outside muscle revealed that for both species the plate-frozen samples were preferred to brine-frozen samples at the first sampling period but this preference declined as storage time increased until at the later sampling periods the brine-frozen samples were slightly preferred over the corresponding plate-frozen ones (Table 4). The significance of the higher salt content in brine-frozen samples in influencing these results is not known, but it is tempting to speculate that it may have masked, or at least modified, the flavor of brine-frozen samples.

For both inside and outside muscle samples the percentage of 'no preferences' tended to increase with length of frozen storage (Table 4).

CORRELATIONS

Total FFA and TBA values were significantly ($P \leq 0.05$) correlated ($r = +.405$) for Pacific halibut but not for chinook salmon.

Only the TBA values of chinook salmon correlated significantly ($P \leq 0.01$) with the detection of flavor differences ($r = +0.460$). The lack of a significant correlation between FFA and flavor for both species differs from the findings of Peters et al. (1968) who reported a correlation of –0.974 between titratable FFA production and average taste panel scores of refrozen Atlantic cod fillets stored at –18 C for 12 months. This discrepancy may be partly attributable to the difference in storage temperatures (Olley et al. 1969) or a reflection of inherent species differences.

Acknowledgments

The authors gratefully thank Miss M. L. Robinson for assistance with the statistical analyses. This research was financially supported by the Fisheries Research Board of Canada.

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Flesh *p*H, Color, Thaw Drip, and Mineral Concentration of Pacific Halibut (*Hippoglossus stenolepis*) and Chinook Salmon (*Oncorhynchus tshawytscha*) Frozen at Sea[1]

J. R. Botta,[2] J. F. Richards,

Department of Food Science
University of British Columbia, Vancouver, B.C.

and N. Tomlinson

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver 8, B.C.

Botta, J. R., J. F. Richards, and N. Tomlinson. 1973. Flesh *p*H, color, thaw drip, and mineral concentration of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) frozen at sea. J. Fish. Res. Board Can. 30: 71–77.

The effects of brine-freezing and plate-freezing at sea on flesh *p*H, color (Hunter *a*/*b* ratio), thaw drip, and mineral concentration were determined at intervals during subsequent storage of the glazed, polyethylene covered fish at –30 C.

The difference in flesh *p*H between the brine- and plate-frozen samples was not significant for either halibut or salmon and the *p*H generally declined during storage.

The changes in the Hunter *a*/*b* ratio of salmon were similar for both methods of freezing and the ratio generally increased slightly during storage.

The thaw drip of halibut and salmon was less for the brine- than the plate-frozen samples after storage for 9–31 weeks whereas subsequently the brine-frozen samples had approximately equal or greater thaw drip than the plate-frozen. Except for plate-frozen halibut, thaw drip tended to increase with length of storage.

Method of freezing did not significantly affect the potassium concentration of either halibut or salmon. For both species the sodium and chloride concentration of brine-frozen outside muscle was at least two to three times greater than brine-frozen inside or plate-frozen muscle.

Botta, J. R., J. F. Richards, and N. Tomlinson. 1973. Flesh *p*H, color, thaw drip, and mineral concentration of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) frozen at sea. J. Fish. Res. Board Can. 30: 71–77.

Les auteurs ont évalué les effets de la congélation par la saumure et sur plaques en mer sur le *p*H de la chair, la coloration (rapport *a*/*b* de Hunter), l'exsudat de décongélation et la teneur en minéraux. Ils ont effectué des mesures périodiques au cours de l'entreposage à –30 C du poisson givré et recouvert de polyéthylène.

Il n'y a pas de différence significative dans le *p*H des chairs d'échantillons de flétan et de saumon congelés en saumure ou sur plaques. En général, le *p*H diminue au cours de l'entreposage.

Les variations du rapport *a*/*b* de Hunter chez le saumon sont semblables pour les deux méthodes de congélation. En général, le rapport augmente légèrement au cours de l'entreposage.

L'exsudat de décongélation du flétan et du saumon est moindre dans les échantillons congelés en saumure que dans ceux congelés sur plaques, après un entreposage de 9 à 31 semaines. Cependant par la suite, les échantillons congelés en saumure ont un exsudat à peu près égal ou supérieur à celui d'échantillons congelés sur plaques. Sauf pour le flétan congelé sur plaques, l'exsudat de décongélation a tendance à augmenter avec la durée d'entreposage.

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[1]This paper is the second in a series of three on the effects of brine-freezing and plate-freezing at sea on the quality of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*).

[2]Present address (J.R.B.): Department of Food Science, Louisiana State University, Baton Rouge, La. 70803, USA.

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La méthode de congélation n'affecte pas de façon significative la teneur en potassium du flétan ou du saumon. Chez les deux espèces, la teneur en sodium et en chlorure du muscle externe est au moins deux ou trois fois plus grande que celle du muscle interne congelé en saumure ou du muscle congelé sur plaques.

Received April 24, 1972

THIS paper is the second of a series reporting the results of an investigation into the effects of brine-freezing and plate-freezing at sea on the quality of frozen stored Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tschawytscha*) (Botta et al. 1973). It deals with the effects of the method of freezing on flesh *p*H, color, thaw drip, and mineral concentration during subsequent frozen storage at −30 C.

Materials and Methods

Catching, freezing, and sampling were described in detail by Botta et al. (1973).

ANALYSIS

Flesh *p*H was determined at several locations throughout the light muscle region of each steak and the readings averaged. Color determinations were conducted on a sample of light muscle taken from the dorsal region of each steak and including portions of the inside and outside regions. Whole steaks were used to determine thaw drip. Mineral concentration was determined in both the first half inch of muscle beneath the skin (outside muscle) and in the next adjacent half inch of muscle (inside muscle).

Flesh *p*H was measured by the method of Tomlinson et al. (1966) using a Metrohm model E 280 A portable *p*H meter.

Hunter '*a*' and '*b*' values were determined on the thawed flesh of chinook salmon using the method of Schmidt and Idler (1958). The reference tile had Rd, '*a*', and '*b*' readings of 8.73, 33.8, and 20.7, respectively.

Thaw drip from the frozen muscle was determined by a slight modification of the U.S. Standard Method for frozen cod fillets (Anon. 1960). The time of thawing at 20 C was 2 hr.

Sodium and potassium concentrations of frozen flesh were determined using the method of Thompson (1969). The dry-ash method was used and the sodium and potassium concentrations in the solutions were detemined using an E.E.L. Flame Photometer.

Chloride concentration was determined using Quantab Titrators S031 No. 1175. The procedure used was similar to that outlined in the instructions for Quantab Chloride Titrators S041 No. 1176 except a total volume of 50 ml was used.

The flesh *p*H, color, thaw drip, and mineral concentration data were statistically analyzed using a split-plot arrangement of treatments. Pairs were considered random and other effects were considered fixed in all analyses. The thaw drip values, expressed as percentages, were transformed, using the arcsine transformation, before any analysis of variance or correlations were conducted. The absolute differences in *p*H, color, and thaw drip values between the brine-frozen and the plate-frozen samples were correlated with the results of the taste panel evaluation (Botta et al. 1973) expressed as the number of correct identifications.

TABLE 1. Analyses of variance of flesh *p*H, thaw drip and Hunter *a*/*b* ratio by species. (Significance at 5 and 1% levels indicated by * and **, respectively.)

		Mean squares				
		Pacific halibut		Chinook salmon		
Source	df	*p*H[a]	Thaw drip[b]	*p*H[a]	Thaw drip[b]	*a*/*b* ratio[a]
Pairs	2	1.26	34.29	3.37*	0.51	14.22
Method[c]	1	0.59	4.39	0.34	0.74	3.75
Error a	2	0.96	48.93	0.20	1.41	3.34
Time	4	2.65*	4.85	5.74**	11.38**	7.08
M × T	4	0.10	13.58**	0.57	3.23	1.32*
Error b	16	0.60	3.52	0.77	1.11	0.29

[a]Mean square values multiplied by 10^2.
[b]Analyses conducted on the arcsine transformed percentages. Mean square values multiplied by 10^4.
[c]Error a was used to test methods. Error b was used to test all other terms.

Results and Discussion

Flesh pH

Analysis of variance of the *p*H data revealed no significant differences in flesh *p*H between brine- and plate-frozen samples of either Pacific halibut or chinook salmon (Table 1). However, significant differences in *p*H related to different storage times for halibut ($P \leq 0.05$) and for salmon ($P \leq 0.01$) were found. The flesh *p*H of halibut decreased steadily during storage (Fig. 1) and was significantly lower ($P \leq 0.05$) at 81 weeks than at 45, 31, and 14 weeks (Table 2). The flesh *p*H of salmon decreased markedly from the 27th through the 58th week and subsequently increased (Fig. 1). The flesh *p*H at 58 weeks was significantly lower ($P \leq 0.05$) than the *p*H at other storage times.

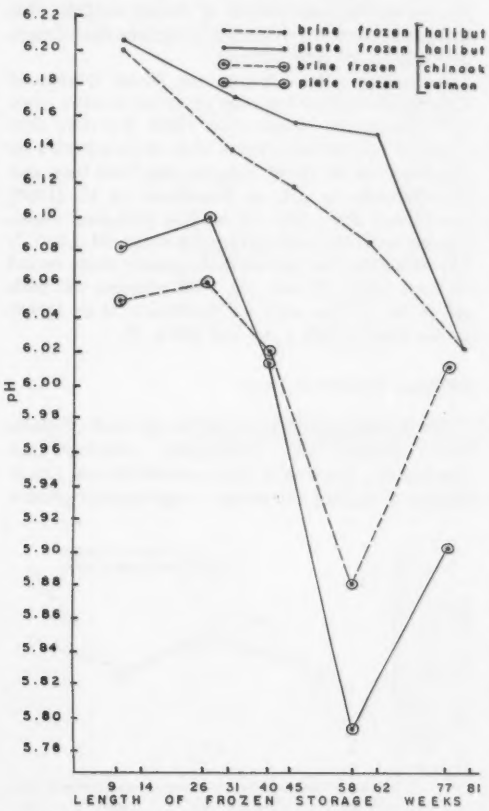

FIG. 1. Average flesh *p*H of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) stored at –30 C.

TABLE 2. Storage time means and results of Duncan's New Multiple Range Test for flesh *p*H and thaw drip by species. Means are presented only where the storage effect was found significant ($P \leq 0.05$) in the analyses of variance. Means not sharing the same letter are significantly ($P \leq 0.05$) different from each other.

Pacific halibut		Chinook salmon		
Weeks of storage	Mean flesh *p*H	Weeks of storage	Mean flesh *p*H	Mean thaw drip (%)
14	6.20^{a}	9	6.08^{a}	6.5^{a}
31	6.16^{a}	26	6.07^{a}	9.0^{bc}
45	6.14^{a}	40	6.02^{a}	9.1^{bc}
62	6.12^{ab}	58	5.97^{a}	7.8^{b}
81	6.02^{b}	77	5.84^{b}	10.0^{bc}

The difference in *p*H between brine- and plate-frozen halibut was small enough that brine-freezing likely would not increase the incidence of the undesirable *p*H-related chalky condition in halibut (Tomlinson et al. 1965a).

The decline in *p*H during frozen storage of Pacific halibut agrees with the findings of Tomlinson et al. (1969). However, in both cases sampling was confounded with position along the length of the halibut and Tomlinson et al. (1966) observed that the *p*H of halibut tends to be higher near the head of the fish and lower near the center of the body. Thus, the observed decline in *p*H may have been influenced by the position of sampling.

Although no information could be found in the literature regarding variation in flesh *p*H along the length of chinook salmon, a positional effect cannot be ruled out.

Flesh Color (Hunter a/b Ratios)

A significant ($P \leq 0.05$) interaction of the freezing method and storage time effects on Hunter *a*/*b* ratios for chinook salmon precluded testing of method or time effects (Table 1). However, between 26 and 77 weeks of frozen storage, the brine-frozen samples had a consistently lower mean *a*/*b* ratio than the plate-frozen (Fig. 2). Except for the period between 26 and 40 weeks of storage the *a*/*b* ratios of both the plate- and brine-frozen samples increased with length of storage (Fig. 2).

Hunter Rd, '*a*', and '*b*' color readings on raw salmon flesh have, by themselves, little practical meaning. However, Schmidt and Idler (1958) found that the '*a*' reading alone was a suitable measure of the color of processed salmon and that it could be predicted from the *a*/*b* ratio of the raw flesh:

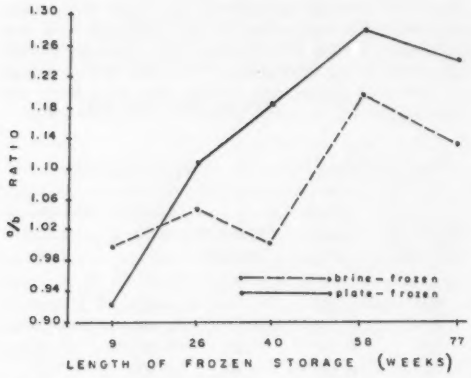

FIG. 2. Average Hunter *a*/*b* ratios of chinook salmon (*Oncorhynchus tshawytscha*) stored at –30 C.

the higher the *a*/*b* ratio of raw flesh, the higher the '*a*' value of the resulting processed product and the higher the visual redness.

Oxidative rancidity of frozen red salmon is accompanied by a fading of the red pigments of the flesh (Tarr 1947, 1955; and Boyd et al. 1957). Although the TBA values indicated oxidative rancidity during early stages of storage (Botta et al. 1972) the *a*/*b* ratio during the corresponding period did not decline (Fig. 2). Thus, the degree of oxidative rancidity apparently was not sufficient to induce measurable pigment fading.

THAW DRIP

The mean thaw drip from plate-frozen halibut, which was greater than that of brine-frozen, decreased during storage while the latter increased and became the greater at 45, 62, and 81 weeks (Fig. 3). This qualitative difference in pattern of change during storage resulted in a highly significant ($P \leq 0.01$) method × time interaction (Table 1).

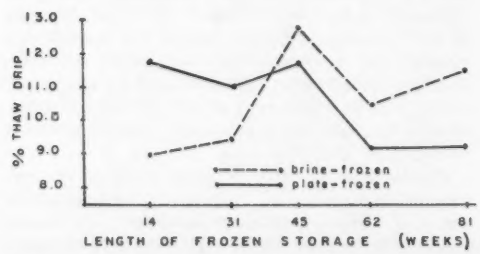

FIG. 3. Average percent thaw drip of Pacific halibut (*Hippoglossus stenolepis*) stored at –30 C.

With chinook salmon the thaw drip at 9 weeks was significantly lower than at other storage times (Table 2, Fig. 4). Freezing method had no significant effect on thaw drip (Table 1).

Fish frozen slowly tend to form more drip than those frozen quickly and the amount of drip increases with increasing frozen storage time (Miyauchi 1963). Also Tarr (1942) showed that NaCl can markedly reduce the amount of free drip formed from frozen fish muscle. The brine-frozen halibut were frozen over a 47-hr period and the salmon were brine-frozen over a 9.5-hr period, whereas both the halibut and salmon were plate-frozen in approximately 3.5 hr. Thus, the fact that the brine-frozen halibut and salmon had less thaw drip than the plate-frozen samples, during the early part of frozen storage, may be due to an NaCl induced reduction of drip. The higher thaw drip of the brine-frozen samples during the later periods of storage suggests that freezing rate may have gradually become the primary determinant of drip volume.

A decline in thaw drip during frozen storage of plate-frozen halibut has been observed in other work with this species (Roach et al. 1966). The thaw drip values of halibut were higher than those reported by Tomlinson et al. (1969) but this may have been due to differences in *p*H, as Tomlinson et al. (1966) found that thaw drip of halibut increased continuously with decreasing *p*H in the range *p*H 6.8–5.7. The flesh *p*H of the halibut in the present study varied between *p*H 6.25 and *p*H 5.85 whereas the flesh *p*H of the halibut used by Tomlinson et al. (1969) varied between *p*H 6.66 and *p*H 6.25.

MINERAL CONCENTRATION

The potassium concentration in the flesh of plate-frozen halibut was consistently, although not significantly, lower than that of brine-frozen. Inside muscle of halibut contained a significantly greater

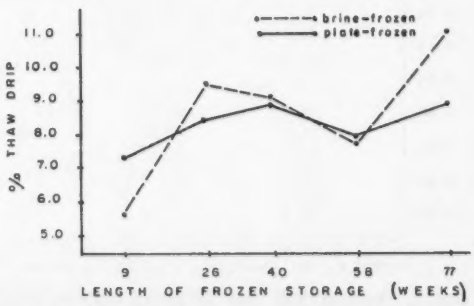

FIG. 4. Average thaw drip of chinook salmon (*Oncorhynchus tshawytscha*) stored at –30 C.

TABLE 3. Analyses of variance of mineral concentration of Pacific halibut and chinook salmon muscle. (** Significant at the 1% level.)

		Mean squares					
		Pacific halibut			Chinook salmon		
Source	df	Sodium	Potassium	Chloride	Sodium	Potassium	Chloride
Pairs	2	0.45	0.48	0.01	0.03	0.07	0.09
Method[a]	1	1.98	0.07	10.66	0.45	1.18	1.33
Error a	2	0.47	0.40	0.05	0.01	0.42	0.11
Location	1	2.35	2.22**	12.67	1.02	2.08**	3.80**
M × L	1	1.65	0.16	9.03**	0.50**	0.00	0.92
Error b	4	0.44	0.05	0.05	0.01	0.03	0.15

[a]Error a was used to test methods. Error b was used to test all terms except methods.

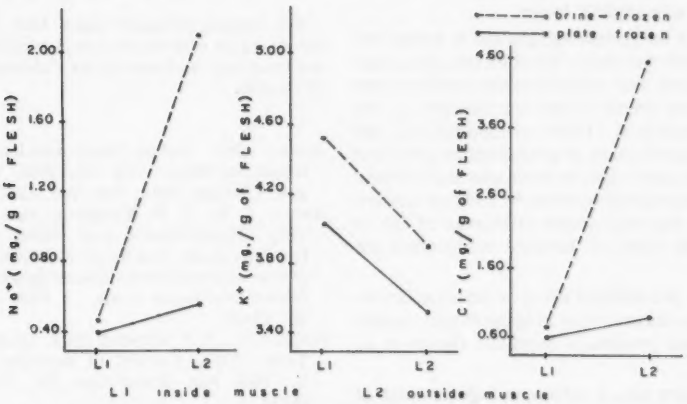

FIG. 5. Average sodium, potassium, and chloride concentration in the flesh of Pacific halibut (*Hippoglossus stenolepis*).

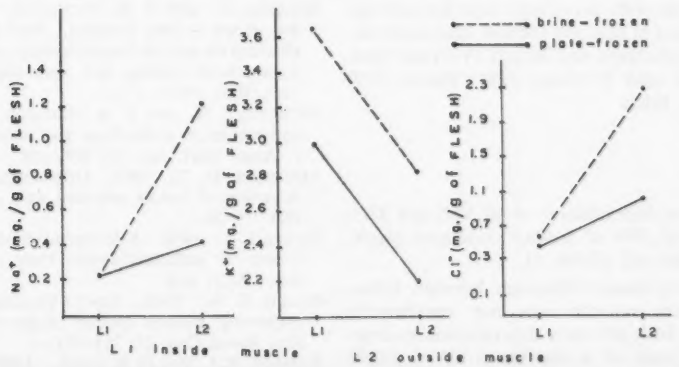

FIG. 6. Average sodium, potassium, and chloride concentration in the flesh of chinook salmon (*Oncorhynchus tshawytscha*).

concentration of potassium than outside muscle (Fig. 5, Table 3).

The difference between brine and plate-frozen halibut in concentration of both sodium and chloride was markedly affected by location. The mean concentration of these minerals was approximately four times greater in brine-frozen outside muscle than in muscle from the other three treatment combinations (Fig. 5).

The effects of freezing method and location on mineral concentrations of chinook salmon were qualitatively similar to those for Pacific halibut (Fig. 6, Table 3).

The sodium concentrations in the brine-frozen inside, plate-frozen inside, and plate-frozen outside muscle of both species were similar to those reported by McBride and MacLeod (1956) but potassium concentration was slightly lower.

The fact that the percentage change in potassium in outside muscle was much less than the percentage change in sodium and chloride concentrations may be related to the length of time the fish were in the brine. Tomlinson et al. (1965b, c) reported that the decrease in concentration of potassium in coho and sockeye salmon and rainbow trout was much slower than the corresponding increase in sodium concentration during the early stages of storage of fish in refrigerated sea water or fortified refrigerated sea water.

The possible pro-oxidant effect of the exceptionally high sodium concentration in brine-frozen outside muscle has been previously discussed (Botta et al. 1673).

Although there was a relatively high content of sodium and chloride in brine-frozen outside muscle of both species, the salt concentration was still below the 1% generally accepted as the palatability limit, and agrees with previously reported findings of 1% or less salt in chinook salmon, chum salmon, and gray cod (Harrison and Roach 1953) and haddock (Holston and Pottinger 1954; Peters 1959) frozen in 23% brine.

Correlations

Only the thaw drip values ($r = -0.358$) and TBA values ($r = +0.389$) of halibut correlated significantly with flesh *p*H (Table 4).

The degree of flavor difference between brine- and plate-frozen samples was not significantly correlated with flesh *p*H, thaw drip or color readings with the exception of a significant ($P \leq 0.05$) correlation ($r = -.527$) between flavor and Hunter '*b*' value in chinook salmon.

TABLE 4. Simple linear correlation coefficients (*r*) between flesh *p*H and each of color, thaw drip, TBA values, and total FFA concentration of Pacific halibut and chinook salmon. (* Significant at the 5% level; ns = not significant at the 5% level.)

Variable	Pacific halibut	Chinook salmon
Color (*a*/*b* ratio)	–	–0.103 ns
Thaw drip	–0.358*	0.074 ns
TBA value[a]	0.389*	0.246 ns
FFA[a]	–0.076 ns	0.072 ns

[a]Mean values for these variables are presented in the first paper of the series (Botta et al. 1973).

Acknowledgments

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Flesh Concentration of Various Long-Chain Free Fatty Acids of Pacific Halibut (*Hippoglossus stenolepis*) and Chinook Salmon (*Oncorhynchus tshawytscha*) Frozen at Sea[1]

J. R. Botta,[2] J. F. Richards

Department of Food Science
University of British Columbia, Vancouver, B.C.

and N. Tomlinson

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver 8, B.C.

Botta, J. R., J. F. Richards, and N. Tomlinson. 1973. Flesh concentration of various long-chain free fatty acids of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) frozen at sea. J. Fish. Res. Board Can. 30: 79–82.

Changes in the concentration of long-chain free fatty acids were determined gas chromatographically from inside and outside muscle samples of brine- or plate-frozen Pacific halibut and chinook salmon stored for 9–81 weeks.

With both species the concentration of individual free fatty acids was greater in outside muscle than in inside muscle. Few differences in concentration of free fatty acids were noted between plate- and brine-frozen samples. Numerous free fatty acids increased significantly in concentration during frozen storage. For both species the increase was most rapid during the first 26–45 weeks of storage. The detection of flavor differences was unrelated to the concentration of individual free fatty acids.

Botta, J. R., J. F. Richards, and N. Tomlinson. 1973. Flesh concentration of various long-chain free fatty acids of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) frozen at sea. J. Fish. Res. Board Can. 30: 79–82.

Nous avons mesuré par chromatographie en phase gazeuse les variations de concentration des acides gras libres à longue chaîne (FFA) sur des échantillons de muscle interne et externe de flétan du Pacifique et de saumon chinook congelés par la saumure ou sur plaques et entreposés durant 9 à 81 semaines.

Chez les deux espèces, la teneur en acides gras libres individuels est plus élevée dans le muscle externe que dans le muscle interne. La teneur en FFA ne présente que très peu de différence entre les échantillons congelés par la saumure ou sur plaques. La concentration de plusieurs acides gras libres augmente sensiblement au cours de l'entreposage réfrigéré. Chez les deux espèces, l'augmentation est plus rapide durant les 26 à 45 premières semaines d'entreposage. La détection de différences dans la saveur est indépendante de la concentration d'acides gras libres individuels.

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While investigating the effects of brine-freezing and plate-freezing at sea and length of subsequent frozen storage on chemical, physical, and organoleptic properties of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*) (Botta et al. 1973a, b), the flesh concentration of various long chain FFA (free fatty acids) was determined. The relationship of total free fatty acid concentration to sensory differences in quality was examined in the preceding articles (Botta et al. 1973a, b). This article describes changes in the concentration of individual long-chain free fatty acids and their relationship to flavor differences.

[1]This paper is the third in a series of three on the effects of brine-freezing and plate-freezing at sea on the quality of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*).

[2]Present address: (J.R.B.): Department of Food Science, Louisiana State University, Baton Rouge, La. 70803, USA.

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Materials and Methods

The catching, freezing, and sampling of the fish are described in detail by Botta et al. (1973b). Briefly, three in-rigor fish of each species were frozen in a plate freezer or in 13% NaCl brine and after glazing were stored in polyethylene bags at –30 C. Inside and outside muscle samples (as diagrammed by Botta et al. 1973b) were removed from each fish at five intervals during the 77–81-week storage period.

The lipids were extracted from 20 g of frozen flesh by the method of Bligh and Dyer (1959). Phospholipids were separated from the total lipids using a procedure similar to that of Hornstein et al. (1967). Neutral lipids (cholesterol, cholesterol esters, mono-, di-, and triglycerides) were separated from the phospholipid-free fraction by the procedure of McCarthy and Duthie (1962) and the 200 ml effluent stored for subsequent determination of neutral lipid content. Free fatty acids retained on the column were removed by two 10-ml aliquots of BF_3–MeOH reagent, containing 125 g BF_3 per 1000 ml MeOH. Methyl esters were prepared from the FFA by mildly refluxing the eluted solution for 15 min. The methyl esters were then recovered by a procedure similar to that of Metcalfe and Smith (1961) except the methyl esters were extracted using 10 ml of *n*-pentane and 20 ml of distilled water and were re-extracted from the aqueous–methanol fraction with a further 5 ml of *n*-pentane. The combined organic fractions were then dried with anhydrous sodium sulfate. The water-free organic fraction was then transferred to a 15 ml test tube, the anhydrous Na_2SO_4 rinsed well with two 3-ml portions of *n*-pentane, and the pentane fractions combined. The volume in the test tube was reduced (using nitrogen gas), transferred to a 1-dr vial, the air space in the vial was flushed with nitrogen gas, the vial immediately sealed at atmospheric pressure, and then stored at –30 C for subsequent gas chromatographic analysis.

The neutral lipid content was determined by flash evaporation of the 200 ml neutral lipid fraction almost to dryness, using a Buchler Flash-Evaporator with the water bath temperature at 30 C. The contents were then transferred to a preweighed weighing dish and dried to constant weight in a vacuum oven at 70 C for 3 hr.

The isolated free fatty acid methyl esters were analysed using a Micro-Tek Model 220 gas chromatograph equipped with a flame ionization detector. The column used was of stainless steel, 10 ft long and $\frac{3}{16}$ inch outside diameter, packed with 3% EGSP-Z (an ethylene glycol-succinic acid diphenyldiethoxysilane polyester) on 100–120 mesh Gas-Chrom Q, Lot 4303, purchased from Applied Science Laboratories. Nitrogen at 5.7 ml/min. was employed as the carrier gas. The inlet temperature was 250 C and the detector temperature was 240 C. All analyses were conducted isothermally with a column temperature of 185 C. Methyl tricosanoate (23:0) purchased from Applied Science Laboratories was used as an internal standard. The fatty acid methyl esters were identified by comparison of their retention times with those of herring oil (Ackman 1966) and cod liver oil (Ackman and Burgher 1965). The recorder was equipped with a Disc Chart Integrator and quantitation was based on the assumption that detector response was proportional to carbon content. The results were expressed as micrograms FFA per gram of neutral lipid.

The long-chain FFA concentration data were statistically analyzed using a split-split plot arrangement. Pairs were considered random whereas all other effects were considered fixed (Steel and Torrie 1960).

Results and Discussion

Plate-frozen halibut contained significantly ($P \leq 0.05$) more free fatty acids 16:0, 17:0, and 20:5 than the corresponding brine-frozen samples (Table 1). With chinook salmon there were no significant differences in individual free fatty acid concentration between the two methods.

In general, with both species, the concentration of the individual free fatty acids was greater in the outside muscle (Botta et al. 1973b, fig. 1) than in the inside muscle (Table 1). This may be the result of a greater concentration of total lipid in the dark than in the light muscle as has been reported in Atlantic halibut (Olley et al. 1962), Atlantic cod (Bligh and Scott 1966), and Baltic herring (Bosund and Garrot 1969).

For halibut the concentrations of FFA 14:0, 16:0, 16:1, 16:2, 17:0, 18:0, 18:1, 18:2, 18:3, 18:4, 20:1, 20:2, 20:4, 22:1, 22:5, and 22:6 significantly increased during storage (Table 1). With salmon the concentrations of FFA 14:0, 15:0, 16:0, 16:1, 16:2, 17:0, 18:0, 18:1, 18:2, 20:1, 20:2, 20:4, 20:5, 22:1, and 22:6 significantly increased during frozen storage (Table 1). For both species the concentration of the free fatty acids increased rapidly during the first 26–45 weeks of frozen storage (Table 1).

Varesmea et al. (1969) observed that with rainbow trout stored for 8 months at –18 C or at –32 C the percentage of fatty acid 18:1 decreased from 23% to approximately zero while the percentage of fatty acid 18:0 increased from 6% to approximately 24%. Olley et al. (1969) showed that with haddock and lemon sole there was a preferential hydrolysis of 16:0, 18:1, and 20:5 phospholipids. In the present study with halibut the concentration of FFA 16:0 and 18:1 but not 20:5 significantly increased during frozen storage (Table 1) and with chinook salmon the concentration of FFA 16:0, 18:1, and 20:5 significantly increased during frozen storage (Table 1). However, with both species the concentration of FFA 18:0 significantly increased rather than decreased as reported by Varesmea et al. (1969) for rainbow trout (Table 1).

The TBA value of chinook salmon was not significantly correlated with the concentration of any

TABLE 1. Method, location, and storage time means from the analyses of variance of free fatty acids (expressed as micrograms free fatty acid per gram of tissue) of Pacific halibut (*Hippoglossus stenolepis*) and chinook salmon (*Oncorhynchus tshawytscha*). (ns = no significant difference at the 5% level, * = significant at the 5% level, ** = significant at the 1% level.)

	14:0	15:0	16:0	16:1	16:2	17:0	18:0	18:1	18:2	18:3	18:4	20:1	20:2	20:4	20:5	22:1	22:5	22:6
								Pacific halibut										
BF[1]	6.5	0.9 ns	43.7*	16.1 ns	0.9[5]	3.5*	18.6 ns	49.4 ns	3.7 ns	1.2	1.1	14.8	0.6 ns	6.7 ns	32.2*	4.5	5.3 ns	63.1
PF	10.2	1.0	59.4	27.4	1.9	5.8	17.2	61.5	5.6	2.0	3.7	27.8	0.7	5.4	56.9	16.0	6.7	88.0
In.[2]	4.8**	0.7 ns	42.8 ns	13.7*	0.9	3.1*	15.9 ns	39.6*	2.9**	1.0	1.0	14.3	0.5 ns	5.0 ns	33.6*	5.3	4.2*	62.0
Out.	11.8	1.2	60.3	29.9	1.9	6.1	20.0	71.3	6.4	2.2	3.8	28.2	0.9	7.2	55.5	15.2	7.8	89.0
14[3,4]	3.8[a]	0.7[a]	30.4[a]	11.3[a]	0.5[a]	1.9[a]	11.1[a]	31.2[a]	2.0[a]	0.8[a]	0.8[a]	9.7[a]	0.2[a]	3.1[a]	27.4[a]	4.2[a]	2.8[a]	50.2[a]
31	9.0[abc]	1.2[a]	74.1[b]	21.4[ab]	1.9[ab]	5.5[b]	24.1[b]	62.2[b]	5.8[b]	1.3[ab]	1.9[ab]	21.7[b]	1.0[b]	8.4[b]	52.2[a]	10.3[ab]	6.3[b]	99.9[b]
45	10.0[bc]	1.1[a]	52.3[ab]	28.5[b]	2.2[b]	5.6[b]	20.2[b]	64.6[b]	5.6[b]	2.3[b]	3.5[b]	27.6[b]	0.9[b]	6.8[b]	54.2[a]	13.6[b]	7.0[b]	91.5[ab]
62	5.8[ab]	0.9[a]	45.6[a]	19.5[ab]	0.9[ab]	4.2[ab]	18.0[ab]	55.4[ab]	4.8[b]	1.9[ab]	2.5[ab]	20.7[b]	0.6[b]	6.5[ab]	46.6[a]	8.4[ab]	6.8[b]	70.7[ab]
81	12.9[c]	0.9[a]	55.3[ab]	28.3[b]	1.4[ab]	6.0[b]	16.4[ab]	63.8[b]	5.1[b]	1.6[ab]	3.2[b]	26.7[b]	0.7[b]	5.6[ab]	42.3[a]	14.8[b]	7.1[b]	63.4[ab]
								Chinook salmon										
BF[1]	58.4 ns	3.0 ns	183.2 ns	106.2 ns	5.4 ns	14.7 ns	38.5 ns	293.1 ns	25.4 ns	14.1 ns	20.7 ns	50.6 ns	2.6 ns	5.1	160.5 ns	32.4 ns	23.9	95.0 ns
PF	55.0	3.1	185.3	98.5	6.3	13.5	45.1	295.2	26.1	14.5	20.3	57.5	2.6	5.0	156.5	35.2	31.5	135.3
In.[2]	44.0*	2.4*	143.1*	81.7*	4.2 ns	9.8*	31.1**	220.7*	18.8**	10.3[5]	14.8	39.8*	2.3 ns	3.4	122.8*	24.8*	20.3	90.8*
Out.	69.4	3.8	225.4	123.0	7.5	18.3	52.5	367.5	32.8	18.3	26.2	68.2	2.9	6.7	194.3	42.8	35.1	139.5
9[3,4]	20.9[a]	1.6[a]	101.2[a]	39.5[a]	1.7[a]	4.1[a]	19.6[a]	122.6[a]	7.0[a]	5.1	5.5	19.2[a]	0.6[a]	2.9[a]	70.3[a]	13.7[a]	12.3	53.6[a]
26	43.3[b]	1.8[a]	189.6[b]	80.5[b]	7.3[c]	12.1[b]	29.6[a]	220.4[ab]	29.5[bc]	12.3	16.5	36.7[a]	5.3[b]	5.0[b]	117.1[b]	34.5[b]	19.6	108.6[b]
40	81.8[c]	5.7[c]	216.4[b]	145.8[d]	10.1[d]	21.7[c]	63.4[b]	414.4[c]	31.9[bc]	20.7	28.9	77.1[b]	2.5[c]	5.9[b]	222.2[c]	45.7[c]	38.2	150.6[c]
58	59.0[b]	3.5[b]	187.9[b]	107.7[bc]	4.1[b]	12.6[b]	44.7[c]	328.8[bc]	23.0[b]	17.3	23.7	62.5[b]	2.3[c]	5.5[b]	160.4[b]	34.3[b]	29.5	105.6[b]
77	78.6[c]	2.8[ab]	226.2[b]	138.1[cd]	6.0[abc]	19.9[c]	51.8[bc]	384.3[c]	37.6[c]	16.1	27.8	74.5[b]	2.3[c]	5.8[b]	222.6[c]	40.9[bc]	38.9	157.4[c]

[1]BF = brine-frozen, PF = plate-frozen. Each mean is the average of 30 observations

[2]In. = inside muscle. Out. = outside muscle. Each mean is the average of 30 observations.

[3]Weeks of frozen storage. Each mean is the average of 12 observations.

[4]Storage time means sharing the same superscript letter are not significantly ($P \leq 0.05$) different from each other by Duncan's New Multiple Range Test.

[5]The absence of lower case letters or asterisks within a set of method, location, or storage means indicates that interactions precluded meaningful statistical tests of the effect (Botta 1971).

of the individual free fatty acids[3]. The TBA value of Pacific halibut flesh was correlated with the concentration of each of nine free fatty acids but the strength of the relationships were weak, yielding coefficients of determination (100 r^2) of less than 33%.

The detection of flavor differences was unrelated to the concentration of free fatty acids in either species.

It should be noted that FFA 22:6 and 24:1 are not separable on a EGSP-Z column (Ackman 1966). Consequently, the FFA designated as 22:6 probably contains 5–10% of FFA 24:1 depending on the species (Gruger et al. 1964).

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Effects of Bunker C Oil on Intertidal and Lagoonal Biota in Chedabucto Bay, Nova Scotia

MARTIN L. H. THOMAS

Department of Biology
University of New Brunswick, Saint John, N.B.

THOMAS, M. L. H. 1973. Effects of Bunker C oil on intertidal and lagoonal biota in Chedabucto Bay, Nova Scotia. J. Fish. Res. Board Can. 30: 83–90.

In February 1970, a large spill of Bunker C oil occurred in Chedabucto Bay, Nova Scotia. The incident was of particular interest since large spills of this type of oil had not previously been studied. Further interest was added by the unusually cold temperatures and by the nonuse of detergents in cleanup. The effects of the oil on intertidal and lagoonal biota have been followed since the accident. Many rocky shores and lagoons were heavily oiled. On exposed shores, oil has decreased steadily since oil stopped coming ashore in mid-1970 and by August 1971 only small amounts remained. In sheltered areas, particularly lagoons, heavy oil contamination remains. The summer remobilisation and subsequent redeposition of oil added a chronic aspect to the pollution. Initial effects of oil involved minor smothering of fauna and tearing loose of algae. Longer term effects involved extensive mortalities of *Fucus spiralis* on rocky shores and *Mya arenaria* and *Spartina alterniflora* in lagoons. Other biota were not visually affected. In all three affected species, mortalities took place either continuously or only in the second year of pollution. Causes of death are unknown. It is recommended that in all intertidal areas very heavy oil deposits should be mechanically removed and the remainder of the oil left to natural degradation.

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En février 1970, il se produisit un déversement considérable d'huile lourde de type "C" dans la baie Chédabouctou, Nouvelle-Ecosse. L'incident présentait un intérêt particulier du fait que des déversements d'envergure d'hydrocarbures de ce type n'avaient pas encore été étudiés. Des températures exceptionnellement froides et la non-utilisation de détergents dans les opérations de nettoyage ont fourni un intérêt additionnel. Depuis l'accident, on a suivi de près les effets de l'huile sur les communautés biotiques de la zone intertidale et des lagunes. Sur les rivages exposés, l'huile a diminué graduellement parce que l'huile a cessé de venir à la côte vers le milieu de 1970, et en août 1971, il n'en restait que très peu. Dans les endroits abrités, surtout dans les lagunes, il y a encore forte contamination. Le dégagement et la redéposition subséquente qui se produisent au cours de l'été donnent à la pollution un aspect chronique. Au début, l'huile lourde a eu pour effet de suffoquer légèrement les animaux et de détacher les algues. Par la suite, il y eut mortalités considérables de *Fucus spiralis* sur les rivages rocheux et de *Mya arenaria* et *Spartina alterniflora* dans les lagunes. A première vue, les autres biota n'ont pas semblé affectés. Chez les trois espèces affectées, les mortalités se sont produites soit de façon continue, soit seulement au cours de la deuxième année qui a suivi le déversement. Les causes de la mort ne sont pas connues. Nous recommandons que les plus grandes accumulations d'huile lourde soient enlevées mécaniquement dans toutes les zones intertidales, et que le reste y soit laissé pour se décomposer naturellement.

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THE tanker *Arrow* carrying 2,800,000 gal of Bunker C oil went aground on Cerberus Rock in Chedabucto Bay, Nova Scotia, on February 4, 1970. Details of the disaster and characteristics of the oil are given in the Report of the Task Force — Operation Oil (Anon 1970). More than half the cargo was spilled into the water, mostly within the first 2 weeks. Much of this oil was driven by prevailing winds on to shores along the northern side of the bay. In this area, as shown in Fig. 1, shorelines are deeply indented and mainly rocky. A small number of sand and gravel shores do occur and there are extensive areas of shallow, muddy lagoons (Owens 1971).

The intertidal biota had not been intensively studied before the catastrophe but evidently was

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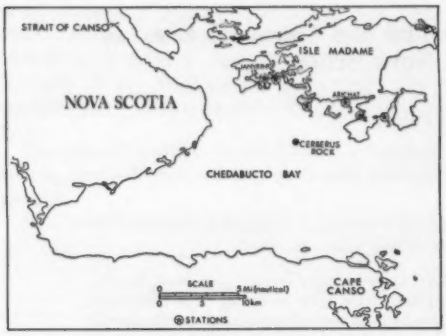

FIG. 1. The Chedabucto Bay area of Nova Scotia showing the general study area and the locations of stations.

typical of that described for this general area by Stephenson and Stephenson (1954) and Bousfield (1960).

Observations on intertidal areas began February 7, 1970, and during the next few weeks included most of the bay. Shores in the Isle Madame area (Fig. 1) were most seriously contaminated; oiling was heavy and repeated. Most other areas were either only lightly oiled or were protected by ice. On the basis of these general observations, seven sites in the Isle Madame area were selected for continuing study. Five were on rocky shores and two in lagoons.

Materials and Methods

Study Sites and Sampling Dates

Fig. 1 shows locations of study stations. On rocky shores the sites which took the form of transects were as follows: Station 1, Janvrin Is. causeway (S. side); Station 2, S. shore of Creighton Is.; Station 3, Arichat Harbour at Arichat Church; Station 4, Arichat Harbour at Petit Barachois; Station 5, Near Petit de Grat. The two lagoonal stations were as follows: Station 6, N. side of Janvrin Lagoon; Station 7, S. side of Janvrin Lagoon.

Sampling dates were as follows: 1970, Mar. 2–5 (stations 3 and 6 only), Apr. 21–22, May 24–25, June 22–23, Aug. 3–4, Nov. 7–8; 1971, Apr. 9–10, Aug. 2–3, 1972; May 25–26.

Transects of Rocky Shores

At each station, a 2-m wide transect at right angles to the shore line was marked at its upper limit by using fluorescent paint on bedrock or a large boulder. Marks were applied at 1-m intervals to each transect for each set of observations.

The following observations were made on each visit to each transect: (1) Zonation of 10–13 common biota by measurement of their upper and lower limits; (2) Abundance of the four algae, *Fucus spiralis*, *F. vesiculosus*, *F. serratus*, and *Ascophyllum nodosum* in 1-m^2 quadrats at selected locations; (3) Photographs of each entire transect and of details at selected locations; (4) General observations on the condition, abundance and distributions of biota; (5) Zonation of oil by % coverage. Estimated in quadrats in the field and by reference to photographs.

At stations 1 and 3, barnacles (*Balanus balanoides*) were counted and photographed at one location.

At station 2 a collection of the common periwinkle (*Littorina littorea*) was made.

Lagoonal Stations

Transects were run as for rocky shores and certain additional observations were made.

At station 6, 1 m^2 of sediment from M. L. W. (mean low water) was dug 25 cm deep and screened through a 4-mm mesh sieve. Benthos were identified and their fresh weight biomass determined. A second quantitative sample of 0.1 m^2 was dug 5 cm (vertical) below E. L. W. (extreme low water). From this sample, eelgrass (*Zostera marina*) shoots were counted and their condition observed.

At station 7, a 0.1-m^2 sample was dug at M.L.W. in a softshell clam (*Mya arenaria*) bed. Clams collected were counted, measured individually, and the entire sample weighed fresh. To the side of the transect, four samples of clams were dug from locations of graded surface oiliness. At each location samples were taken at random except for avoiding previous sample sites. The clams collected were carefully examined and classified as normal, moribund (alive but unreactive), newly dead (body remains present), or dead (joined shells with entire ligaments). Surface oil cover and oil penetration down and laterally from burrows was checked at each sample site. Occasionally, living clams were held in running, oil free sea water in the laboratory and subsequent mortalities and oil ejection noted.

Photographs proved to be of particular value in the comparison of locations at different dates.

Results

General Observations

By February 7, 1970, when observations commenced, many shores in the Isle Madame area were heavily coated with oil and tidal pools were filled. Much of the intertidal biota was oil coated or covered. In oily areas, masses of mixed oil and algae (mostly *Fucus serratus*) were washed ashore. The scarcity of stranded algae on nonoiled shores suggested that oil was implicated in the breaking away of this alga.

During the few months following the grounding, wider areas became heavily coated with oil. At the same time previously oiled areas were often recontaminated.

Reoiling is still occurring in Chedabucto Bay mostly in summer when stranded oil is remobilised by higher temperatures. This repeated reoiling adds a chronic element to the pollution.

Shore water temperatures in winter fall to about −1 C and in summer rise to about 18 C on exposed shores and 22 C in lagoons.

Fish Mortalities

On February 26, 1970, dead fish (4 species) were observed stranded and washing ashore in the general area of station 1 (Fig. 1). Two counts of dead specimens were made with the following results:

	Count 1	Count 2
Ulvaria subbifurcata (Radiated shanny)	6	2
Myoxocephalus scorpius (Shorthorn sculpin)	5	16
Liparis atlanticus (Seasnail)	2	10
Pholis gunnellus (Rock gunnel)	1	0
Total	14	28

Count 1 was on 30 m of shoreline on Janvrin Island Causeway and count 2 on 400 m of the southwest shore of Janvrin Island.

Continued sporadic mortalities were observed in this area, but not elsewhere, up to March 4, 1970.

All the species involved were of shallow-water demersal habit. External examination revealed no cause of death and no oil. The area involved was not only heavily oiled but also the site of many experiments in shore cleanup using both chemical and physical methods. Because of this complication the cause of death could not be determined.

Transects of Rocky Shores (Stations 1–5)

Oil cover and zonation — Generally the percentage of rocky shore areas coated with black oil was greatest at about M.H.W. (mean high water), decreasing to zero at E.L.W. and also above high tide level. Overall coverage was at a maximum between February and July 1970 and then declined steadily. Typical situations are illustrated for stations 2 and 3 in Fig. 2A, B. Increases in coverage shown for the summer of 1970 reflect redistribution of oil. Oil cover at M.H.W. reached a maximum of 100% whereas at M.L.W. it never exceeded 50%. Declines at all levels have been steady since July 1970.

A somewhat more complex pattern is shown in Fig. 2C, which illustrates events on the sheltered Petit Barachois transect (Station 4). Oil splashed at E.H.W. (extreme high water) did not greatly decrease from June 1970 to August 1971. The initially very heavily oiled upper shore showed close to 100% cover until November 1970 when a steady decline began. The lower shore behaved similarly to other locations.

Most areas showed most rapid declines in oil cover during the winter of 1970–71; this was attributed to rough weather and ice scour.

Intertidal biota — Most of the approximately 20 species of biota observed, with the exception of that smothered by heavy oil, appear to have been little affected. Barnacles (*Balanus balanoides*) showed no unusual mortalities and settlement of larvae occurred in 1970, 1971 and 1972. Periwinkles (*Littorina saxatilis*, *L. littorea*, and *L. obtusata*), the commonest intertidal animals, are still abundant and occupy generally the same zones. A minor contraction in the zone occupied by *L. obtusata* was attributed to algal mortality discussed below.

Fucus spiralis, an alga forming a narrow zone at about M.H.W., has been reduced or eliminated at locations where oiling was severe. The decline of this species at three stations is shown in Fig. 3. There was no evidence of recolonisation except at one location during the summer of 1972. There the new plants were minute, and identification impossible; they died during the following winter. *F. spiralis* did not die abruptly when oiled but declined gradually in the 10 months or so following initial oiling.

Observations in Lagoons

Oiling and oil zonation — Oiling in lagoons was heavier and more persistent than on rocky shores. These features acted as natural oil traps. During the first few months following the grounding, layers of oil up to several centimeters in thickness were common and on one occasion over 30 cm was measured. Wave and ice-scour action is negligible in lagoons and natural attrition of oil has been slow. Intertidally, coverage by oil or mixed oil and eelgrass leaves was usually complete, some of the oil mixing with the upper part of the sediment to form a firm pavement-like layer. The situation at Station 6 was typical and is illustrated in figure 2D. The upper intertidal area showed complete oil coverage throughout the period of observation. The upper and lower fringes of this zone both showed complete oiling in early 1970 but an erratic decline since then. The upper fringe still showed over 50% cover in May 1972. Redistribution of lagoonal oil has been a continuing intermittent feature and is responsible for fluctuations in oil cover. As shown in Fig. 2D,

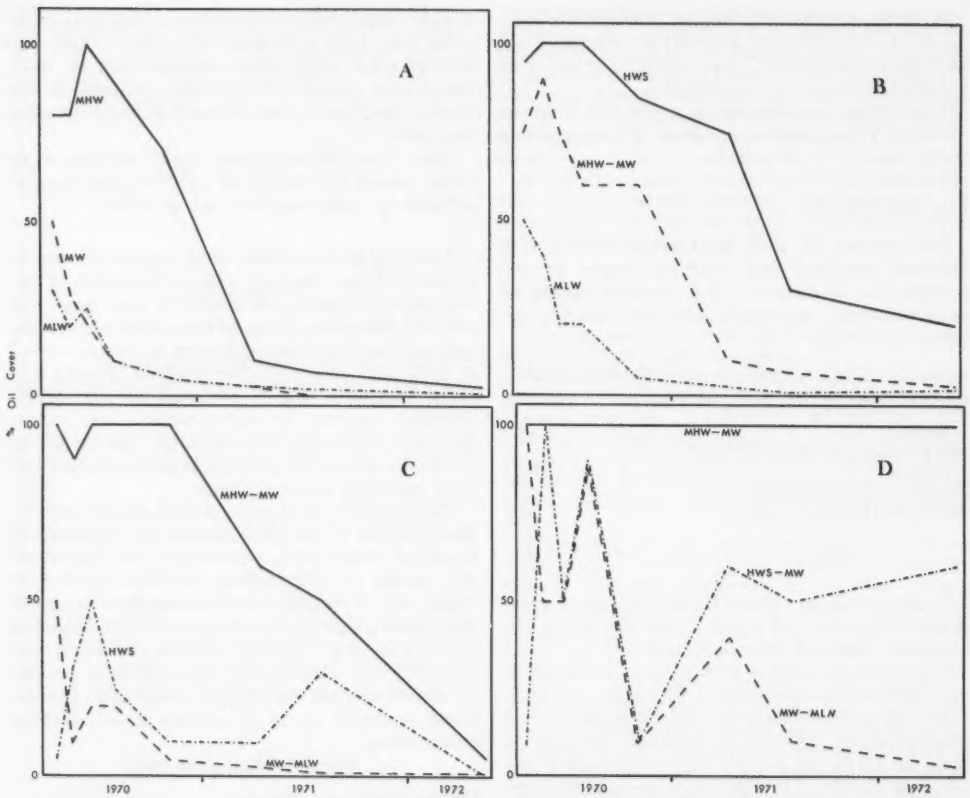

FIG. 2. Shore oil cover at stations 2–4 (A–C) and 6 (D). Lines show percentage oil cover at various tidal heights or in tidal zones. Abbreviations are: HWS, high water spring tides; MHW, mean high water; MW, mean water level; MLW, mean low water.

these fluctuations are most marked in summer when oil is most fluid.

Subtidally, in the shallow water eelgrass beds, considerable quantities of oil became incorporated in the sediments, perhaps forced there by ice. Disturbance of this sediment results in the release of oil droplets to the surface.

Effects on biota — S. alterniflora or salt-marsh cord grass grows abundantly on the sheltered shores of lagoons above midtide level. This species has been eliminated in some very heavily oiled areas. In some such locations it failed to grow in 1970, in others spikes of leaves were able to penetrate the oil and oily sediment, and grew well in 1970, but failed to do so, or did so in greatly reduced abundance in 1971. Additionally, spikes that grew in 1971 were small and chlorotic. Evidently the effects of oil on this species were more severe in the second year following oiling.

Mya arenaria, the softshell clam, is abundant in the area, particularly in the lagoons. Mortalities of this species were first observed when the ice melted in 1970 and have continued sporadically since but in general have been proportional to the amount of oil cover. Results for Station 7 are illustrated in Table 1. In this table, the relation between mortality and oil cover is most clearly shown by comparison of total mortality (calculated from samples, assuming no recruitment) with mean oil cover for the entire period of observation. The table also presents data on moribund clams. This condition was apparently mainly associated with early mortalities only and this may indicate a changing cause of death.

In addition to the mortality of clams in the sediment reflected in the results above, a second cause of mortality was observed. In early 1970 when oiling was most severe, many clams were observed to vacate their burrows. The exposed clams were largely eaten by local predators. Examination of clam burrows revealed that many of these had a pool of oil below the clam shell. It appeared that clams let oil entering the burrow bypass the shell and then moved up above accumulated oil. In this way they could eventually reach the surface. This phenomenon did not persist possibly because hardening oil prevented clam surfacing.

Clams were visibly contaminated with oil and taste panels reported strong oily flavour. Because of this and because many clams were moribund and dying, clam fishing was closed in the area and remains closed at the time of writing.

Clams removed from the area and held in clean sediment in running oil-free sea water in Prince Edward Island ejected visible oil for at least one month following transfer.

The quantitative samples of eelgrass (*Z. marina*) collected at Station 6 have shown no significant changes in abundance, growth pattern, or flowering, although considerable oil is present in the sediment.

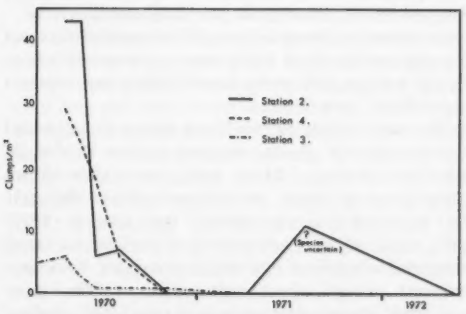

FIG. 3. Abundance of *Fucus spiralis* at mean high water level at Chedabucto Bay shore stations.

Discussion

There are several almost unique aspects to the *Arrow* oil spill that make the results of special significance. Firstly, few large spills of Bunker C have been reported and none have had extensive research programs associated with them. Secondly, the water temperature at the time of the grounding and for several subsequent months was at or close to freezing and ice was present in sheltered locations. These facts make results applicable to arctic spills in some respects. A third peculiar aspect was that detergents were not used to any extent. Most investigations of the effects of oil spills on biota were complicated by detergent use. (Smith 1968; Simpson 1968; Carthy and Arthur 1968).

Although these unique aspects enhanced the value of collected data, an opposite effect was introduced by the general lack of information on the biological characteristics of the area prior to the spill. Chedabucto Bay is relatively isolated and has not been the site of any detailed work on the biology of shore areas. This situation contrasts with that of several British Isles spills of crude oil for which excellent "before and after" information was gathered. (Smith 1968; Carthy and Arthur 1968; Cowell and Baker 1969).

The behavior of oil on the shores of Chedabucto Bay contrasted strongly with the reported behavior of other oils (mostly crude) elsewhere. Most of these

TABLE 1. Percent of dead and moribund (figures in brackets) *Mya arenaria* at Janvrin Lagoon, Station 7.

Date	Plot No. 1	2	3	4
May 5, 1970	8.0(28.0)	25.9(22.2)	38.5(46.1)	12.9(5.6)
May 25	– (7.4)	– (7.4)	– (23.1)	(0.0)
June 23	– (0.0)	– (0.0)	– (0.0)	– (0.0)
Aug. 4	12.6 (0.0)	– (0.0)	21.9 (0.0)	1.9(0.0)
Nov. 7	31.3 (0.0)	31.5 (0.0)	25.0 (0.0)	– (0.0)
Apr. 10, 1971	– (0.0)	3.2 (0.0)	– (0.0)	– (0.0)
Aug. 3	11.4 (0.0)	20.6 (0.0)	17.7 (7.4)	1.4(0.0)
May 26, 1972	2.7 (2.7)	9.7 (9.7)	10.3 (5.2)	4.8(2.4)
Total Mortality	52.4	64.7	73.4	19.8
Mean Oil Cover %	51.6	74.0	85.2	1.5

differences can be attributed to the nature of the oil and to the cold temperatures. On Chedabucto Bay shores, Bunker C oil was deposited in heavy layers which adhered tenaciously to rock, algae, and ice. In places thick coatings remained for extended periods and in such locations there has been no reestablishment of populations originally smothered. Although the oil weathered to a fairly firm surface, this process involved only a thin skin and, during subsequent warmer temperatures, oil from beneath the weathered layer softened and started to move. These weathered surfaces were unstable and often recoated with oil. Heavy coatings of oil and quantities of oil trapped in stranded plants also no doubt contributed to the high incidence of reoiling from shore reservoirs, a phenomenon still occurring over 2 years after the grounding. The instability of weathered oil and the repeated reoilings have injected a chronic aspect to the pollution not reported elsewhere. Except where crude oil is dammed on shores, it apparently forms fairly thin layers and weathers to a stable surface (Smith 1968; Simpson 1968; Carthy and Arthur 1968; Nelson-Smith 1968; Mitchell et al. 1970; Spooner 1970; Stebbings 1970).

This aspect of the behavior of Bunker C oil on shores suggests that every effort should be made to clean up stranded oil reservoirs as soon as possible and to repeat the operation as remobilized oil causes recontamination.

No doubt the mobility of the oil and the thick layers involved slowed down natural physical attrition of the oil. However, the results show clearly that, at least in all but very sheltered areas, oil disappeared fairly rapidly from rocky shores. This and the fact that mortalities of rocky shore biota were generally small fortify the advice of many biologists that rocky shore areas should be left without treatment except where really deep oil makes mechanical removal necessary (Smith 1968; Simpson 1968; Baker and Crapp 1970). Certainly environmental damage would be less than if detergents or steam cleaning were used (Smith 1968; Simpson 1968).

It was notable that the rafts of mixed seaweed and oil that stranded soon after the grounding were predominantly of *F. serratus*, which occurs in the lower intertidal zone. Midtide seaweeds, such as the dominant *A. nodosum* or *F. vesiculosus*, were not prominent in rafts although they would be exposed to the most severe oiling. It is assumed that these species are protected by a mucilage layer which prevents oil adhesion. Such an action has been described for other species (Jones et al. 1969; Mitchell et al. 1970).

Unfortunately fixed littoral epifauna, such as barnacles, are not abundant in the area, probably due to harsh winter conditions and ice scour (Stephenson and Stephenson 1954; Thomas 1970). These species would give better evidence of the immediate effects of oil than the dominant intertidal fauna, periwinkle species. Periwinkles would no doubt be washed away if dead or inactive. However, the complete absence of all fauna from very heavily oiled shores suggests immediate inactivation or death. North et al. (1964) reported similar results from an area heavily contaminated with diesel fuel. Very heavy oiling would inevitably involve smothering except for a few shellfish that can respire anaerobically.

Another early event, that of the limited fish kill, cannot, as explained above, be certainly attributed to Bunker C oil. It seems likely that deaths were caused by chemicals introduced as solvents or ignition agents. Less likely as a cause are detergents. The toxicity of both these general groups of compounds to fish is well documented (Mitchell et al. 1970; Smith 1968; Tarzwell 1970; Gooding 1971; Simpson 1968; Hampson and Sanders 1969).

In summary, immediate and observable effects of the oil on intertidal biota were only severe where oiling was exceptionally heavy, elsewhere observable effects were small.

The later effects on biota are better documented and possibly of greater interest, but on the whole were not obvious. Most fauna on rocky shores showed no evidence of adverse effects. Barnacle (*B. balanoides*) larvae settled and grew in 1970, 1971, and 1972. The dominant periwinkles have remained abundant and their zonations have not changed except where seaweed mortalities have occurred. These observations support the contentions of authors such as George (1971), Spooner (1970), and Crapp (1971a,b) that relatively heavy fractions of oils are not highly toxic to intertidal animals. Ottway (1971), however, reports that, in laboratory tests, these heavy fractions are toxic.

The eradication of the upper intertidal alga *F. spiralis* was the sole example of extensive mortalities on rocky shores. It is curious that the plants died so slowly; this also suggests low immediate toxicity of the oil. North et al. (1964) found evidence that diesel oil caused very rapid mortalities in intertidal algae. In England, mortalities of this species were recorded following the *Torrey Canyon* spill (Nelson-Smith 1968; Smith 1968) but all instances were complicated by detergent use. The Chedabucto Bay evidence shows that *F. spiralis* will be killed by prolonged exposure to oil alone. Its high intertidal position makes it very vulnerable as natural attrition is slowest there.

Effects on the intertidal biota were most marked in sheltered lagoons that had initial heavy oiling and

repeated summer reoiling. But, even there, extensive mortalities were observed in only two species, the softshell clam (*M. arenaria*) and the salt marsh cord grass (*S. alterniflora*).

Ice may well have prevented observations on initial mortalities in clams since these were evident when ice receded in May 1970. As explained above, early mortalities involved both deaths within the substratum and deaths of clams leaving their burrows. This latter effect has been observed with other oil spills (Jones et al. 1969; North 1967; Spooner 1970) but involved different infaunal species. The reason for this behavior has not been explained but observations in Chedabucto Bay suggested it was an avoidance reaction to pooled oil in burrow bottoms. It is also possible that oil reduced oxygen levels in burrows by sealing burrow mouths, by reducing gaseous exchange between air and sediments, and by promoting bacterial action. Infaunal bivalves tend to leave their burrows when oxygen is depleted.

Many clams, however, died in their burrows and are continuing to do so; the results showed that mortalities were proportional to the percentage of oil cover on adjacent sediments. The cause of mortality has not been ascertained but it was shown that clams contained oil and retained it over long periods. If it is toxic, as shown by Ottway (1971), death could be by slow poisoning. It may also be associated with interference with feeding. Smith (1968) and George (1970) have reported that oils interfere with cilliary feeding mechanisms and therefore energy acquisition. By mechanisms discussed above, oxygen lack may also be involved and escape behavior may be prevented by hardening surface oil and sediment layers. The actual cause of mortality should be investigated in laboratory experiments.

The mortalities of salt marsh cord grass (*S. alterniflora*) were not unexpected since such effects have been reported before in connection with both oil and detergents (Baker 1970, 1971a, b, and c; Cowell 1969). Baker (1970, 1971a, b, and c), who has made extensive studies on the effects of oil on salt marshes, rates *Spartina* as having intermediate susceptibility to oil. She found that it could stand a single oiling but declined rapidly if repeatedly oiled. In general Baker found that this perennial recovered rapidly from oil pollution and in fact formed an efficient oil trap. The trapping action was particularly efficient in winter when no living shoots occur above the ground. In Chedabucto Bay, oiling was in effect repeated and occurred at all times of the year. Baker (1970) attributes *Spartina* death to lethal effects of increased reducing conditions in the sediments to the plant's rhizomes; however, elsewhere (1971e) she points out that oxygen will normally diffuse to the roots internally if stems and leaves are not oiled. In Chedabucto Bay, mortalities were most noticeable during the second winter of oil contamination. It seems likely that conditions for *Spartina* may well be critical in winter when aerial shoots are absent. Especially critical conditions would be expected during a winter following a summer of heavy contamination, since plants would enter winter in an abnormally reducing sediment. Possibly, then, mortalities may be indirect but Baker (1971d, e) has also shown direct toxic effects.

Where rhizomes survive, recovery of *Spartina* is rapid but when rhizomes die only slow recovery can be expected. Stebbings (1970) looked at salt marshes in Brittany following the *Torrey Canyon* disaster and again 16 months later. On the second investigation he found that recovery of *Spartina* was virtually complete. However, investigation showed that new plants were growing entirely in new sediment on top of oil. Roots were absent below oil. This suggests that recovery may well be more rapid in areas of rapid sedimentation rate. In Chedabucto Bay observations indicate negligible accretion of new sediment in *Spartina* stands. Recovery may well be slow.

In Chedabucto Bay the most abundant lagoonal biota were softshell clams and salt marsh cord grass. Both were adversely affected by Bunker C oil. However, cleanup by other than mechanical means cannot be advised. Adverse effects of detergents or emulsified oils could only be accentuated by shallow water and limited exchange. Despite the slow attrition of soil in such locations, leaving oil to natural degradation seems the only logical approach. As on rocky shore areas, very thick oil should be mechanically removed. The removal of stranded oiled plant remains is especially important in such areas as they form natural traps for such material.

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Measurement of Formaldehyde in Fish Muscle Using TCA Extraction and the Nash Reagent

C. H. Castell and Barbara Smith

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

Castell, C. H., and B. Smith. 1973. Measurement of formaldehyde in fish muscle using TCA extraction and the Nash reagent. J. Fish. Res. Board Can. 30: 91–98.

The use of the Nash test, in conjunction with TCA extraction, for measuring formaldehyde in fish muscle was made more quantitative. This was done by means of a "recovery factor" which took into consideration the percent of formaldehyde added to the muscle extracted by the TCA solution. The average recovery from 15 different samples of cod muscle was 51.3% with an SD of 5.6. Because of differences between species in capacity to bind formaldehyde, it would appear that a different "recovery factor" may be required for each species of fish. As heating muscle increases its ability to bind formaldehyde, recovery factors developed for use with raw muscle are not applicable to the same muscle after it has been cooked. The percentage of added formaldehyde that was recovered varied with variations in the procedures used in preparing the muscle, making the extract, and carrying out the Nash test.

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Le test de Nash, couplé à l'extraction au TCA, pour le dosage du formaldéhyde du muscle de poisson a été rendu plus quantitatif. Nous nous sommes servis pour cela d'un "facteur de récupération" qui tient compte du pourcentage de formaldéhyde total dans le muscle soumis à l'extraction par la solution de TCA. La récupération moyenne observée sur 15 échantillons différents de muscle de morue est de 53.1%, avec écart type de 5.6. Par suite de différences dans leur capacité de fixation du formaldéhyde, chaque espèce de poisson semble exiger un "facteur de récupération" différent. Le chauffage du muscle augmente sa capacité de fixation du formaldéhyde. Pour cette raison, les facteurs de récupération établis pour le muscle cru ne s'appliquent pas au même muscle après cuisson.

Nous avons également étudié l'effet des variations de procédures dans la préparation du muscle, la fabrication de l'extrait et la conduite du test de Nash sur la proportion de formaldéhyde ajouté qui peut être récupérée.

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A procedure for the estimation of formaldehyde in biological materials was described by Nash (1953). It is based on the formation of diacetyldihydrolutidine from acetylacetone and formaldehyde in the presence of excess ammonium salt. He suggested (but did not support it with data) that the test could be carried out without the complete destruction of the biological material being examined. He went even further by stating "the conditions are mild enough to allow of its use with living material, and its sensitivity and degree of specificity are comparable to those of other reactions requiring more severe conditions." A careful reading of his paper shows that this proposed procedure is simply a colorimetric method for measuring free or loosely bound formaldehyde. Formaldehyde, however, reacts readily, and in some cases almost irreversibly, with proteins, amines, amino acids, and many other organic compounds. In biological materials much of this bound formaldehyde is not available to react with the Nash reagent; therefore, this test is not a satisfactory measure of the total amount of formaldehyde that is present in plant or animal tissues unless it is used in conjunction with some method of extraction that releases the more firmly bound formaldehyde. In most analytical procedures this is accomplished by the very methods that Nash suggested were not required, such as the complete destruction of the tissues by means of hot, concentrated sulphuric acid, as used by Eegriwe (1937). However, this drastic treatment poses a difficulty when applied to the tissues of many marine animals. Soudan (1962) has pointed out that strong acid treatment also liberates formaldehyde from trimethylamine oxide, which is a normal constituent of most sea fish. This oxide, therefore, has to be elim-

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inated or reduced before the test can be carried out.

Amano and Yamada (1964) attempted to adapt the Nash test to the measurement of formaldehyde in fishery products by a much gentler method of extraction. They first macerated the fish in a mortar and then extracted it with a 20% TCA solution, but they gave no data on the efficiency of the extraction. Somewhat later, Amano and Tozawa (1969) made the following significant comment: "Because of its highly reactive nature with proteins, or with many other components in fish tissue, a full determination of formaldehyde may not be feasible "(i.e. by means of the Nash test on a TCA extract).

The purpose of this present work was to re-examine the procedure for measuring formaldehyde by means of TCA extraction and the Nash test. An attempt was made to estimate the proportion of the total formaldehyde present in fish muscle that can be recovered by TCA extraction. This was done by adding measured amounts of formaldehyde to samples of fish muscle, measuring the amounts recovered in the TCA extract, and then calculating the percentage of the added formaldehyde that was recovered. If this "recovery factor" is reasonably constant for muscle from a given species of fish, it would then be possible to convert the results of the Nash test on a TCA extract into values that more closely approximate the total amount of formaldehyde in the tissues. As the formaldehyde-binding capacity of muscle is primarily determined by its chemical composition, and as the composition of muscle varies slightly from one species to another, it is anticipated that a different "recovery factor" may be required for each species of fish. Most of the work described in this paper was confined to fresh cod muscle and to concentrations of formaldehyde not exceeding 0.01% or 100 ppm in the muscle. A study was also made of the effects of variations in the procedures used for preparing the muscle, making the extract, and carrying out the Nash test on the amount of formaldehyde that was recovered. In addition, some tests were carried out to determine the formaldehyde-binding effect of certain components of the muscle, as indicated by their ability to change the proportion of formaldehyde that was recovered when they were added to the muscle.

Materials and Methods

Most of the experimental work was carried out with muscle from Atlantic cod (*Gadus morhua*), but for the purpose of comparison tests were also made with muscle from Atlantic haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*), silver hake (*Merluccius bilinearis*), Atlantic wolffish (*Anarhichas lupus*), redfish (*Sebastus marinus*), winter flounder (*Pseudopleuronectes americanus*), Atlantic salmon (*Salmo salar*), and lobster (*Homarus americanus*). Most of the fish were obtained live from the station's aquaria and the tests were commenced before the fish had entered rigor mortis. A number of tests were also made with fillets obtained from commercial sources that were of good quality but which had passed through rigor.

The formaldehyde was analytical reagent grade. The basic procedures for the experimental work were as follows:

(1) Because of the difficulty of obtaining uniform distribution of formaldehyde when added to whole fillets, blended fillets were used. The blend was prepared by blending a whole fillet with an equal weight of water in a gallon size Waring blendor for approximately 45 sec at medium speed.

(2) The formaldehyde solutions were always prepared from the stock 40% solution immediately before being used. They were diluted in such a manner that the desired concentration was obtained when one part of formaldehyde solution was mixed with 2 parts of blend by weight. The final blend then contained 1 part of fish to 2 parts of water and 60 ppm of formaldehyde. All results are recorded as ppm (mg/1000 g) in the blend.

(3) After standing 30 min at room temperature, the blended muscle, containing the formaldehyde, was extracted by adding an equal weight of 10% TCA solution, stirred, and filtered. A series of comparative tests showed no difference in the amount of formaldehyde recovered from blends by using 10% TCA, 10% $HClO_4$, or plain water. In a typical test the recoveries were: 52.7% with TCA, 49.2% with $HClO_4$, and 52.0% with water. The disadvantage in using water was that it required extra centrifuging to remove the cloudiness that developed during the heating of the reaction tubes, which could not be eliminated by filtering. We also found no advantage in increasing the strength of the TCA solution from 10% to the 20% used by Amano and Yamada.

(4) Nash color reagent: A solution containing 2-M ammonium acetate, 0.05-M acetic acid, and 0.02-M acetylacetone was prepared by dissolving 150-g ammonium acetate, 3 ml of acetic acid, and 2 ml of acetylacetone (redistilled at 140 C) in water and adjusting the volume to 1000 ml. This solution was stored in a dark colored, airtight bottle at 0 C.

In reference to the stability of his color reagent, Nash (1953) stated that after 2 weeks at 20 C it "appeared to be unchanged." Our experience has shown it to be less stable than indicated. Even when stored close to 0 C the optical density values with known amounts of formaldehyde gradually decreased as the storage period increased. Cochin and Axelrod (1959) overcame this by preparing a slightly modified, double-strength Nash reagent (DSNR): 150-g ammonium acetate and 2 ml of acetylacetone in a volume made up to 500 ml with water. We found that when the DSNR was stored 6 months at 0 C it gave readings that were identical to those from freshly prepared reagents. Tests using both DSNR and freshly prepared Nash reagent gave similar results. Because of its better keeping quality, DSNR was used in most of the formaldehyde determinations recorded in this paper.

(5) A 5-ml sample of the TCA extract was added to 10 ml of water and adjusted to *p*H 6.0 (with acetic acid

or NaOH solutions) and then made up to 25 ml with water. Five milliliters of this adjusted and diluted extract were added to 5 ml of the Nash color reagent followed by heating in a water bath for 5 min at 60 C and then cooling under running tap water. The optical density (OD) was read at 415 nm and the formaldehyde calculated by comparison of values obtained with a standard curve. During the course of the work three different spectrophotometers were used and compared: Unicam, Beckman DU, and Bausch and Lomb "Spectronic 20." They all gave satisfactory readings, but the "Spectronic 20" needed the use of a line voltage regulator to give steady readings.

Standard Curve and Recovery Factors

The curve obtained by plotting formaldehyde concentrations in aqueous solutions against OD readings, using the Nash color reagent (Fig. 1), shows a straight line relation up to a formaldehyde concentration of about 25 ppm (OD of about 0.7). Over a period of a year, using several lots of separately prepared Nash and DSNR solutions, 45 tests were made on standard solutions containing 20 ppm of formaldehyde. The average OD was 0.513 with an SD of 0.052. From these figures a factor was obtained for the conversion of OD values into formaldehyde concentrations (where the OD values were under 0.7): Each OD unit of 0.1 equals approximately 3.9 ppm of formaldehyde. The relation between the concentrations of added formaldehyde in the blend and the OD values that were obtained by TCA extraction and the Nash test (Fig. 1) was also linear for OD values up to 0.7. Calculations based on the values from which these curves were drawn show that for this particular sample of fish muscle approximately 44% of the added formaldehyde was recovered in the TCA extract.

Formaldehyde Recovery from Cod Muscle

The blend made from the two fillets from one cod fish, containing 60 ppm of formaldehyde, was divided into 10 portions and a formaldehyde determination was made on a TCA extract from each of the 10 portions. The average recovery was 55.6% and the SD was 0.51. Also, over a period of several months a similar concentration of formaldehyde was added to muscle from 15 different cod fish. In this case the average recovery was 51.3% and the SD 5.6. This indicates that, although there might be slight variations between muscles from individual fish, approximately 50% of the formaldehyde added to the cod muscle was recovered in the TCA extracts.

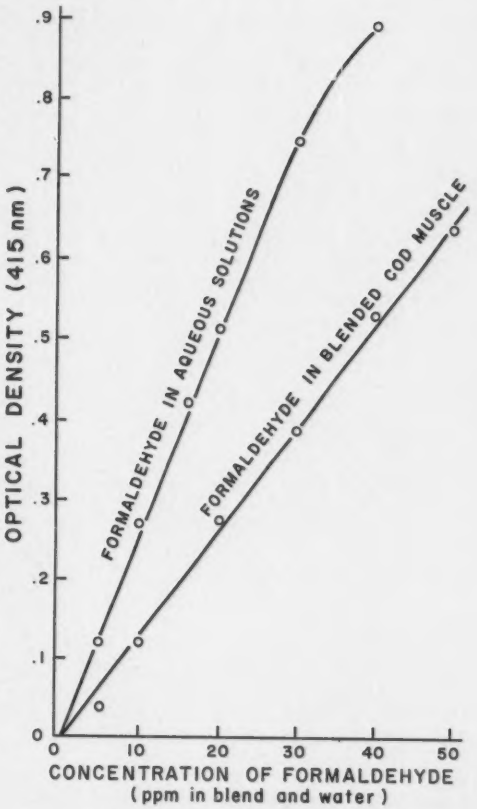

FIG. 1. Standard curve showing the relation between optical density and the concentration of formaldehyde in aqueous solutions, and the curve showing the relation for formaldehyde added to the fish muscle, obtained from the TCA extracts. Each plotted value is the average for five or more samples.

Measurement of Formaldehyde Formed in Galoid Fillets During Storage

In dealing with commercial fish one would rarely be required to measure the amount of formaldehyde that has been purposely added to the muscle. Of much more interest is the formaldehyde that is formed in situ as the muscle undergoes certain types of deterioration or processing. An example of this is the formaldehyde that is formed in the muscle of gadoids when the fillets are frozen and stored at temperatures above –25 C (Tokunaga 1964, 1965; Castell 1971). In this case the formaldehyde has its origin in the trimethylamine oxide (TMAO) of the muscle (Amano and Yamada 1964a, 1964b; Yamada and Amano 1965), which decomposes through enzyme action to form equimolar amounts of DMA and formaldehyde. During storage the DMA accumulates, but at least part of the formaldehyde is firmly bound by the tissues and cannot be recovered by TCA extraction. With this in mind, DMA and formaldehyde (using the copper dithio-carbonate procedure of Dyer and Mounsey 1945) determinations were carried out on some old frozen fillets that had been stored under poor storage conditions. The purpose was to compare the amounts of DMA and formaldehyde that had formed during storage in terms of millimoles per 100 g of muscle. To begin with,

the formaldehyde was measured in the usual manner by TCA extraction and the Nash color reagent, but without the use of a "recovery factor." The average values from four separate fillets obtained in this way were as follows:

	DMA	HCHO
mmole/100 g	0.82	0.44
molar ratio	1	0.536

This would suggest that during storage only half a mole of formaldehyde was formed for each mole of DMA. However, if the average "recovery factor" for cod muscle (Table 1) is taken into consideration, quite a different set of values is obtained:

	DMA	HCHO
mmole	0.82	$\frac{100}{51.5} \times 0.44 = 0.85$
molar ratio	1.00	1.04

indicating that very close to equal molar amounts of DMA and formaldehyde were produced in the stored frozen fish.

Factors Affecting the Recovery of Formaldehyde

This section indicates how some factors affect the amount of formaldehyde that can be recovered from cod muscle.

Proportions of Muscle and Water in the Blends

When the proportions of muscle and water in the blends were changed, there was a corresponding change in the amounts of added formaldehyde that were recovered. As the proportion of muscle increased the amount of recoverable formaldehyde decreased (Fig. 2), indicating that the unrecovered fraction was bound by the tissues.

Binding of Formaldehyde by the Soluble and Insoluble Components of the Muscle

Both the solid and the liquid fractions of the muscle were able to bind the formaldehyde, but the greatest "loss" occurred with the insoluble materials, as shown by the following experiment: A 1:1b lend of cod muscle was centrifuged at 41,300 *g*. The supernatant liquid was drained off by inverting the tubes and letting these stand for 10 min. This gave, by weight, 31% very compact solid material and 69% clear, liquid solubles. The liquid was then divided into two portions, one of which was treated with TCA and filtered to remove soluble proteins and other materials precipitated by the TCA. Thirty-one grams of the solids were made up to 100 g

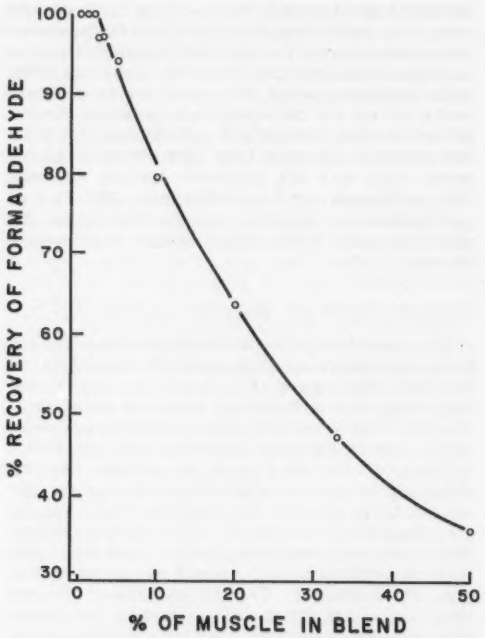

Fig. 2. Effect of the proportions of water and muscle in the blend on the recovery of added formaldehyde. The formaldehyde was added to give a concentration of 60 ppm in each of the blends.

by adding water and dispersed by vigorous stirring. Sixty-nine grams of each of the liquid fractions were also made up to 100 g by the addition of water. To each lot formaldehyde was added to give a concentration of 60 ppm and, after standing for 30 min, they were tested as usual:

Fraction	% Recovery of HCHO
Insolubles	49.2
Solubles	66.3
TCA-treated solubles	69.7

It is noteworthy that removal of the soluble proteins from the liquid fraction by TCA treatment made relatively little difference in its capacity to bind formaldehyde.

Blending Time

Insufficient blending, where the tissues were not completely disintegrated, resulted in a greater recovery of the added formaldehyde; but once the blend had reached a smooth creamy texture more blending

had no effect. With most fish muscle samples this creamy stage was reached after 1–1.5 min at medium speed in a Waring blendor.

Time Between Adding Formaldehyde and Testing

In well blended muscle the "loss" of added formaldehyde occurred rapidly. With samples left for 3–120 min between the addition of formaldehyde and extraction with TCA solution, the percentage recoveries showed no significant differences:

Time (min)	3	5	25	60	120
Recovery (%)	49.7	50.8	51.4	54.1	50.8

Heating the Reaction Tubes

Using DSNR for determining the recoverable amounts of formaldehyde added to blended cod muscle, we found that heating the reaction tubes beyond 5 min at 60 C resulted in decreasing optical density readings (Fig. 3). This agrees with the results obtained by Nash using the ordinary color reagent, in which he found the reaction time for 99% completion of the color change to be 40 min at 37 C or 5 min at 58 C.

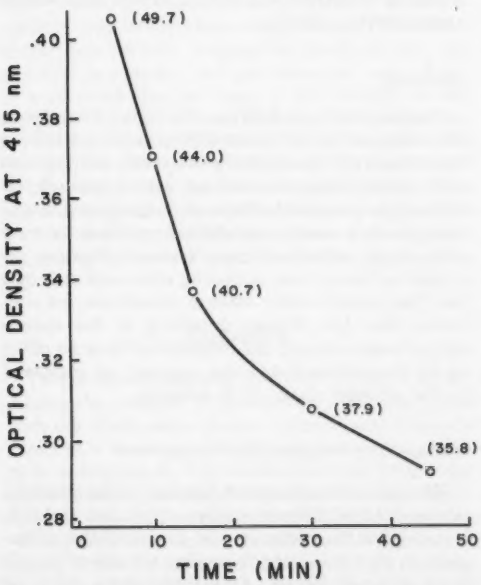

Fig. 3. Effect of holding time at 60 C on color development in reaction tubes containing the double strength Nash reagent and TCA extracts from blended cod muscle to which formaldehyde had been added to give a concentration of 60 ppm. The figures in brackets show the percentage recoveries of formaldehyde.

Trimethylamine and Dimethylamine

In defining some of the limitations of his colorimetric procedure Nash (1953) pointed out that certain amines can compete with the ammonia in the reagent, causing "an apparent loss of determined formaldehyde." As both trimethylamine (TMA) and dimethylamine (DMA) are frequently found in marine fishery products, it is important to know whether either one of these amines interferes with the recovery of formaldehyde from fish muscle. To answer this, cod muscle blends were prepared from freshly killed fish, under conditions where neither TMA or DMA has a chance to develop. TMA–HCl and DMA–HCl were each added to give a range of amine values between 6 and 66 mg of amine nitrogen per 100 g of muscle. These concentrations extend beyond the limits normally encountered in badly deteriorated commercial fish. The blends were tested 30 min after adding the amines and the results showed that in the concentrations used they had little or no effect on the recovery of the formaldehyde:

Concn of amine in blend (mg amine N/100 g)	0	6	12	20	27	33	66
Recovery of HCHO(%)							
With TMA	46	52	47	46	44	46	44
With DMA	46	47	48	49	47	49	41

Other tests in which the *p*H of the blend was adjusted to an alkaline range of *p*H 7.0–9.0 (in order to free the amine from the hydrochloride) gave similar negative results.

Proteins and Amino Acids

The addition of various proteins (casein, gliadin, gluten, zein, and peptone) to give a concentration of 1% in the blends added little or nothing to the normal formaldehyde-binding activity of the muscle itself, as indicated by the amounts recovered in the TCA extract. If much larger concentrations were added, or if the muscle was heated before extracting it with the TCA, additional binding of the formaldehyde became evident. The same remarkable result was obtained with most of the free amino acids that were added to the muscle. One decided exception was with cysteine, and to a somewhat lesser extent with the closely related peptide, glutathione. The effects of cysteine on the recovery of formaldehyde, both from aqueous solutions and from blended cod muscle, are shown in Fig. 4. In the case of the blends, the muscle itself reduced the recovery by approx-

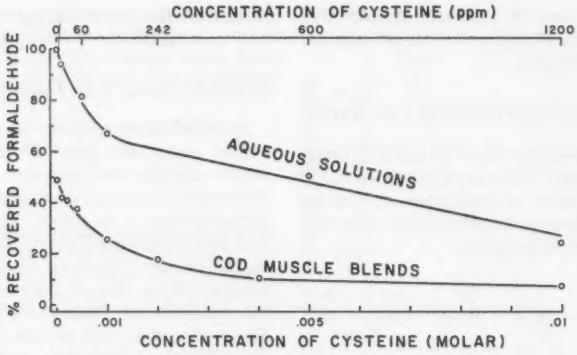

FIG. 4. Effect of cysteine on the recovery of formaldehyde from aqueous solutions and from blended cod muscle.

imately 50%, but the addition of relatively small amounts of cysteine further reduced the amounts recovered. The addition of 60 ppm (0.006%) cysteine was more effective in reducing the recovery of formaldehyde from the blend than the addition of 5% of any of the proteins that were tested.

The results with cysteine were not unexpected. It is known that it combines with formaldehyde to form thiazolidine-4-carboxylic acid, which is a remarkably stable compound towards both acid and alkali (Ratner and Clarke 1937). In addition to reacting with formaldehyde, cysteine also reacts directly with the Nash color reagent, seriously interfering with the results of the test. Fortunately, this can be prevented by precipitating the cysteine in the TCA extract with a weak solution of $CuSO_4$ and filtering immediately before the Nash reagent is added.

Heating the Muscle

Preheating the muscle increased its capacity to bind formaldehyde and therefore decreased the percentage of added formaldehyde that could be recovered. For example: four fillets were cut from two fish. One fillet from each fish was autoclaved for 15 min at 15 lb pressure, while the two corresponding fillets were left unheated. These were each blended separately and formaldehyde was added and extracted in the usual manner. The recoveries were 47.6 and 49.8% from the unheated muscle and 21.6 and 24.0% from the corresponding heated fillets. Heating the blends after the formaldehyde had been added resulted in even lower recoveries than when heat was applied to the whole fillet before it was blended and the formaldehyde added. For example: fillets were blended in the normal manner and the blends were mixed and distributed in heat-stable plastic bags. One was left unheated; a second was heated by immersing it for 10 min in boiling water before adding formaldehyde; the third had formaldehyde added before heating. The recoveries were as follows: Unheated, 56.8%; heated prior to adding formaldehyde, 33.1%; heated after adding formaldehyde, 23.7%.

Freezing

Freezing fresh cod fillets at −40 C for 2 hr and then defrosting and adding formaldehyde did not change the amount of formaldehyde recovered, as compared with similar fillets that had not been frozen. Determining the combined effects of freezing and frozen storage was a much more difficult problem because of the many variables that are involved. However, as a result of many tests, including some with fish that had been stored under adverse conditions, we concluded that the changes occurring in the muscle during frozen storage had relatively little or no effect on its formaldehyde-binding capacity, as indicated by the recovery from TCA extracts.

Intra and Inter Species Differences

Because of the inherent differences in the chemical composition of different species of fish and shellfish, it is possible that there may be corresponding differences in the formaldehyde-binding activity of muscle from different species. As a preliminary check on this problem determinations were made on the percent recovery of formaldehyde added to blended muscle from 10 species of commercial fish and shellfish (Table 1). Although these limited data are insufficient for a satisfactory statistical analysis, they

TABLE 1. Percentage recoveries of formaldehyde[a] that had been added to blends prepared from the muscle of various species of fish and shellfish using the Nash test and 10% TCA as the extractant.

Species	No. of fish	% Recovery	
		Range	Avg
Scallops	7	66.7–71.7	69.1
Lobster	4	59.1–64.1	61.7
Atlantic salmon	1	–	66.9
Winter flounder	2	53.4–54.8	54.1
Cod	15	40.3–58.6	51.5[b]
Haddock	5	44.1–54.7	48.4
Hake	2	45.1–51.3	48.2
Pollock	3	39.7–49.4	43.7
Wolffish	5	33.1–44.2	40.2
Redfish	1	–	39.9

[a]Formaldehyde added to give a concentration of 60 ppm in the blend.

[b]Standard deviation from the mean: 5.6.

do suggest that a species difference may exist. The range of formaldehyde recoveries from the scallops and lobsters was considerably higher than those from the fish. Among the species of fish, the gadoids, as a group, had recoveries that were closer to each other than to those of the shellfish or the nongadoid species of fish. However interesting these results appear to be, they need the confirmation that can only be obtained by a much more extensive set of measurements. It is also very probable that, with some species, the recovery factors may differ with some of the chemical changes that occur in the muscle during the annual cycles of feeding and spawning.

Discussion

This work started out with the object of determining the value of TCA extraction in conjunction with the Nash color reagent (Amano and Yamada 1964) as a measure of small amounts of formaldehyde in fish muscle. The work is far from being completed because so many factors are involved. There are the problems resulting from different species of fish, variations in the composition of muscle resulting from the cycles of spawning and feeding, and the effects of various ways of storing and processing the muscle.

The results do show, however, that the test as it has been used in the past is far from quantitative. However, by determining the percent of formaldehyde added to the muscle that can be recovered by extraction with TCA, a value much closer to the total amount of formaldehyde present can be obtained. It was shown that for added formaldehyde the recovery from cod muscle is approximately 50%. The very limited number of tests with muscle from other species suggests that some at least may differ from cod in their formaldehyde-binding activity, as indicated by extraction with TCA.

Knowing the extreme reactivity of formaldehyde with many compounds it was a little surprising to find that the addition to the muscle of 1% of several different proteins made relatively little change in the recovery of added formaldehyde. But it must be remembered that these small amounts of protein were added to a very complex system already containing proteins and many other compounds with which formaldehyde will combine. In contrast to this, the extremely effective binding of formaldehyde when trace amounts of cysteine were added to the muscle was equally interesting and suggests the need for the examination of other sulphydral compounds in this connection.

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Gill Irrigation and Pressure Relationships in Rainbow Trout (*Salmo gairdneri*)

JOHN C. DAVIS[1] AND DAVID J. RANDALL

Department of Zoology
University of British Columbia, Vancouver 8, B.C.

DAVIS, J. C., AND D. J. RANDALL. 1973. Gill irrigation and pressure relationships in rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 30: 99–104.

In studies on 11 rainbow trout (*Salmo gairdneri*), 217.3±6.5 g, 14.0±0.5 C) fitted with rubber membranes for direct measurement of ventilatory water flow ($\dot{V}_G$), buccal, cleithral, and differential pressure traces increased in amptitude as $\dot{V}_G$ rose from 40 to 160 ml/min. Ventilation rate remained steady at around 77 breaths/min in the $\dot{V}_G$ range 40–120 ml/min and rose to 90 breaths/min at a $\dot{V}_G$ of 175 ml/min. Oxygen uptake more than doubled as $\dot{V}_G$ rose from 40 to 120 ml/min. Calculated utilization of oxygen from the inspired water declined only slightly over this $\dot{V}_G$ range, indicating that large increases in nonrespiratory spillage of water (anatomical dead space) had not occurred. The calculated oxygen cost of breathing was low. Area mean differential pressure appeared closely related to $\dot{V}_G$ over the $\dot{V}_G$ range 40–160 ml/min. Area mean differential pressure increased from two- to four fold over this $\dot{V}_G$ range, depending upon which individual fish was examined. Over the $\dot{V}_G$ range 40–160 ml/min the calculated resistance of the gill sieve did not appear to decline although some variability was present and a variety of gill sieve resistances could be measured at a given $\dot{V}_G$ for each fish. It is concluded that changes in $\dot{V}_G$ are largely dependent on changes in mean differential pressures across the gills, with no marked decline in gill sieve resistance over the normal physiological range of $\dot{V}_G$.

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Au cours d'essais sur 11 truites arc-en-ciel (*Salmo gairdneri*) (217.3±6.5 g, 14.0±0.5 C) munies de membranes de caoutchouc permettant de mesurer directement l'écoulement de l'eau de ventilation ($\dot{V}_G$), le tracé des pressions buccales, cleithrales et différentielles augmente d'amplitude à mesure que $\dot{V}_G$ passe de 40 à 160 ml/min. Le taux de ventitaltion demeure constant à environ 77 respirations/min, sur une gamme de 40 à 120 ml/min de $\dot{V}_G$, et s'élève à 90 respirations/min à un $\dot{V}_G$ de 175 ml/min. La fixation de l'oxygène fait plus que doubler lorsque $\dot{V}_G$ passe de 40 à 120 ml/min. L'utilisation de l'oxygène à partir de l'eau aspirée, telle que calculée, ne diminue que légèrement sur cette gamme de $\dot{V}_G$. Il n'y a donc pas eu d'augmentation considérable d'épanchement non respiratoire d'eau (espace anatomique mort). Le coût calculé en O_2 de la respiration est bas. La pression différentielle moyenne par région semble intimement liée à $\dot{V}_G$ sur une gamme de 40 à 160 ml/min de $\dot{V}_G$. La pression augmente de 2 à 4 fois sur cette gamme de $\dot{V}_G$, suivant le poisson. Sur une gamme de $\dot{V}_G$ de 40 à 160 ml/min, la résistance du filtre branchial ne sembie pas diminuer, bien qu'il existe certaines variations et qu'il ait été possible de détecter, sur chaque poisson, différents degrés de résistance du filtre branchial à un $\dot{V}_G$ donné. Nous concluons que les variations de $\dot{V}_G$ dépendent en grande partie des changements de pression différentielle moyenne à travers les branchies, sans qu'il y ait déclin notable dans la résistance du filtre branchial, sur la gamme physiologique normale de $\dot{V}_G$.

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[1]Present address: Fisheries Research Board of Canada, Pacific Environment Institute, 4160 Marine Drive, West Vancouver, B.C.

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ALTHOUGH the pressure relationships within the branchial pumps of trout are well documented (Hughes and Shelton 1957, 1958, 1962; Hughes and Saunders 1970; Shelton 1970; van Dam 1938) there is little information on the relationship between these pressures and the volume flow of water over the gills. This study was designed to simultaneously measure ventilation volume ($\dot{V}_G$), buccal and opercular cavity pressure and differential (buccal minus opercular) pressure across the gills of rainbow trout. It was hoped that this approach would provide information on changes in resistance of the gill sieve at different $\dot{V}_G$'s.

Methods

Eleven rainbow trout (217.3±6.5 g) were fitted with oral membranes and placed in ventilation boxes as described by Davis and Cameron (1970). The oral membrane, a portion of a surgeon's rubber glove affixed to the margin of the fishes' mouth, physically separated inspired and expired water and allowed collection of the latter. Polyethylene cannulae (Clay Adams, P.E. 190) 20 cm long were implanted in the buccal and opercular cavity as described by Saunders (1961).

Pressures were recorded (Fig. 1) with a Sanborn 267 BC differential pressure transducer and a Beckman Type R Dynograph recorder. Turning the appropriate taps allowed recording of zero, buccal, cleithral, and differential pressure. Equivalent 20-cm lengths of this tubing (0.47-cm bore) were used on opposite sides of the transducer. Cannulae were connected to the large bore tygon tubing by a male/male luer adaptor and the hub of a number 18 gauge spinal needle. Frequency response of the system with the cannula attached, measured using the transient analysis of Yanof (1965, p. 276), was 10.2 cycles/sec.

The experimental procedure was as follows. Fish were cannulated, fitted with oral membranes, and placed in the $\dot{V}_G$ boxes for overnight recovery. The following morning the pressure recording system was set to zero with the water level in the $\dot{V}_G$ box and calibrated by applying a known pressure with a manometer. The cannulae were then connected to the recording system and the fish was left undisturbed for an hour in its black plastic covered box. Buccal pressure, opercular cavity pressure, differential pressure, inspired oxygen tension (PIO_2), expired oxygen tension (PEO_2), and $\dot{V}_G$ (ventilation volume in ml/min) were then recorded at intervals during the day. Differential pressure and $\dot{V}_G$ were always simultaneously recorded and the recorder zero was checked after each pressure measurement. The area mean differential pressure for each fish at each $\dot{V}_G$ was calculated from the area under the differential pressure trace (Fig. 1). As the objective of this study was to obtain pressure/flow data at a variety of $\dot{V}_G$'s, attempts were made to obtain a range of $\dot{V}_G$'s for each fish. Some fish struggled periodically and thus produced a range of ventilatory flows while others had to be stimulated by tapping on the side of the box. Water temperature in the apparatus ranged from 12 to 15.5 C during the study ($\bar{x}$ = 14.0± 0.5 C). Oxygen uptake rate ($\dot{V}_{O_2}$) was calculated from inspired oxygen tension, expired oxygen tension, $\dot{V}_G$ and the oxygen solubility coefficient for the test temperature (Davis and Cameron 1970). Oxygen uptake and utilization were not recorded at high $\dot{V}_G$'s as high $\dot{V}_G$'s were of a transitory nature. They occurred after a fish had been struggling and tended to drop off rapidly following struggling. Thus samples of expired water used to determine $\dot{V}_{O_2}$ would not be representative of a single $\dot{V}_G$ but a range of declining $\dot{V}_G$'s.

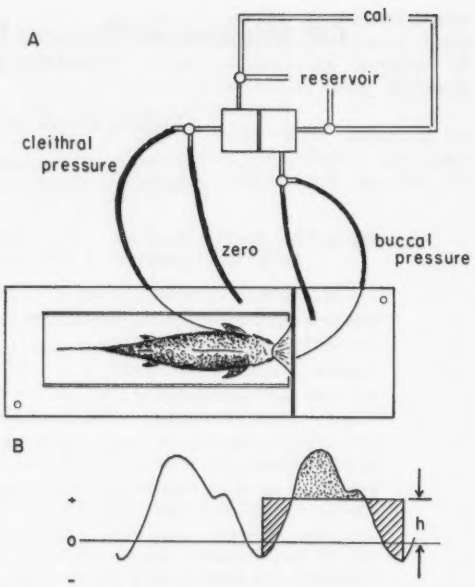

FIG. 1. A, The system used to record buccal, cleithral, and differential pressure from trout with oral membranes attached. Manipulation of the taps allowed recording of buccal pressure, cleithral pressure, differential pressure, or pressure in the water, or from a calibration manometer. B, A hypothetical differential pressure trace showing how area mean pressure (*h*) was determined. The line dividing the cross-hatched and stippled areas is positioned so that the stippled area is equivalent to the sum of the two cross-hatched areas. The positive pressure equivalent to *h* is the area mean pressure (Burton 1966).

Results

At the lowest mean $\dot{V}_G$, 40 ml/min, ventilation rate was 77.3±2.4 breaths/min and Vsv (volume of water pumped/breathing cycle) was 0.59±0.03 ml/breath, while buccal pressure, cleithral pressure

and differential pressure (maximum/minimum) were 0.70/–0.23, 0.62/–0.3, 0.59/–0.39 mm Hg, respectively (Table 1).

Over the $\dot{V}_G$ range 40–120 ml/min these pressures increased gradually. At higher ventilatory flows the amplitude increased appreciably. All the pressures tended to vary considerably from one individual to another and a range of pressures was often recorded at any one $\dot{V}_G$ for an individual fish. Following struggling, pressure rapidly rose to high levels. One or 2 min after attaining this high level, the pressure usually dropped appreciably towards resting levels. Usually the pressure trace contained one brief phase when opercular cavity pressure exceeded buccal pressure. The magnitude of this reversed phase tended to decrease when $\dot{V}_G$ went up. In one indivdual buccal pressure was high at very high $\dot{V}_G$'s, and always exceeded opercular cavity pressure.

A plot of area mean differential pressure vs. $\dot{V}_G$ for each fish gave results similar to those of Fig. 2. Pressures tended to vary somewhat at any one $\dot{V}_G$, particularly at the lower $\dot{V}_G$'s. Mean differential pressure increased between two- and fourfold for each fish over the $\dot{V}_G$ range 40–160 ml/min. Comparisons between individual fish showed considerable variation in the mean differential pressure at any one $\dot{V}_G$ and the extent to which this pressure increased as $\dot{V}_G$ went up. Similar plots of area mean buccal and cleithral pressure vs. $\dot{V}_G$ showed a weak relationship between those pressures and $\dot{V}_G$.

For each area mean differential pressure measurement, at each $\dot{V}_G$, the total resistance (Guyton 1962, p. 344) of the gill sieve was calculated from:

$$R = \frac{1333 \times P}{F}$$

Where: R = resistance in CGS units, a basic physical resistance unit (expressed in dyne sec/cm^5); P = the mean differential pressure in mm Hg; F = the water flow over the gills in ml/sec.

The gill resistance calculated in this way reflected the variation observed in mean differential pressure by nature of the calculation. Over the $\dot{V}_G$ range 40–160 ml/min, however, calculated gill sieve resistance for each fish tended to remain relatively constant (Fig. 2) and did not drop appreciably as $\dot{V}_G$ increased.

Ventilation rate (Table 1) remained around 75 breaths/min over the $\dot{V}_G$ range 40–120 ml/min and increased when $\dot{V}_G$ rose above 120 ml/min.

TABLE 1. Ventilatory characteristics recorded from 11 rainbow trout (217.3±6.5 g) with oral membranes attached (temp. = 14.0±0.5 C). Values are means ± standard errors (brackets). Pressures are in mm Hg.

	$\dot{V}_G$ – ml/min							
Measurement	40	60	80	100	120	140	160	180
Ventilation Rate	77.27	78.43	72.94	75.50	73.79	79.38	89.00	90.50
#/min	(2.44)	(3.41)	(2.42)	(1.32)	(2.08)	(1.85)	(2.86)	(1.43)
Vsv	0.59	0.81	1.14	1.33	1.63	1.76	1.86	1.43
ml/breath	(.025)	(.038)	(.051)	(.023)	(.053)	(.049)	(.047)	(.026)
% U	44.87	55.11	40.50	43.33	42.75	–	–	–
	(3.10)	(1.66)	(10.12)	(3.40)	(3.35)			
$\dot{V}_{O_2}$	62.76	93.37	99.46	138.73	137.78	–	–	–
mg/kg/hr	(4.97)	(4.37)	(36.27)	(12.35)	(13.03)			
Max. buccal	0.70	0.86	0.81	0.89	0.98	1.15	1.53	1.52
pressure	(.071)	(.105)	(.080)	(.061)	(.080)	(.115)	(.291)	(.190)
Min. buccal	–.23	–.37	–.36	–.44	–.40	–.77	–1.02	–1.35
pressure	(.049)	(.064)	(.096)	(.096)	(.089)	(.174)	(.240)	(.295)
Max. cleith.	.621	.647	.554	.634	.659	.679	.940	.820
pressure	(.079)	(.089)	(.094)	(.077)	(.082)	(.101)	(.292)	(.136)
Min cleith.	–.30	–.44	–.56	–.56	–.67	–.75	–.56	–1.21
pressure	(.060)	(.040)	(.087)	(.076)	(.090)	(.082)	(.102)	(.171)
Max. diff.	.591	.731	.836	.767	.841	.995	–	–
pressure	(.090)	(.092)	(.124)	(.142)	(.187)	(.140)		
Min. diff.	–.386	–.370	–.387	–.374	–.335	–.601	–	–
pressure	(.088)	(.075)	(.106)	(.133)	(.188)	(.195)		

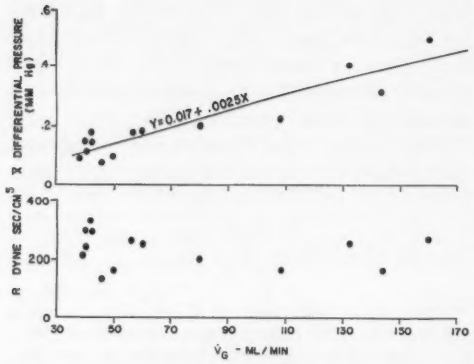

FIG. 2. Area mean differential pressure vs. $\dot{V}_G$ (*upper panel*) for a single 229 g trout at 12.4 C .Values are individual observations. The *lower panel* illustrates the resistance of the gill sieve calculated for each point in the upper panel.

Ventilatory stroke volume, however, rose in a nearly linear fashion between 40–120 ml/min $\dot{V}_G$, reflecting the rising $\dot{V}_G$ with no change in VR. At $\dot{V}_G$'s above 120 ml/min the rate of increase of Vsv in relation to $\dot{V}_G$ declined due to the rising VR. The highest Vsv recorded was 1.93 ± 0.03 ml/breath.

Calculated oxygen uptake rate increased from 62.7 ± 4.9 to 137.8 ± 13.0 mg/kg/hr as $\dot{V}_G$ increased from 40–120 ml/min. Percent utilization at a $\dot{V}_G$ of 40 ml/min was $44.9 \pm 3.1\%$ and showed only a slight decline over the 40–120 ml/min $\dot{V}_G$ range (Table 1).

Inspired oxygen tension remained between 148 and 153 mm Hg in all cases.

Discussion

Though our analysis is in terms of mean differential pressure and the effect of oscillations in flow on the phase relationships between pressure and flow during each breathing cycle has not been measured, these results elucidate a number of important relationships between pressure and flow across the gills of trout. Though mean differential pressure was somewhat variable at a given $\dot{V}_G$, over the $\dot{V}_G$ range of 40–160 ml/min, there appeared to be a close relationship between mean differential pressure and $\dot{V}_G$. Hence, over that $\dot{V}_G$ range, the relationship between calculated gill sieve resistance and $\dot{V}_G$ did not change appreciably.

If we assume that maximum $\dot{V}_{O_2}$ in rainbow trout is eight times resting (Brett 1962) and that percent utilization does not change with $\dot{V}_G$, as observed in this study over a limited range of ventilation volumes, then $\dot{V}_G$ at maximum $\dot{V}_{O_2}$ will be about 320 ml/min at 14 C. The maximum range of $\dot{V}_G$'s recorded for rainbow trout using the oral membrane technique was 26–280 ml/min (Davis and Cameron 1970) and quiescent fish rarely exceeded 150 ml/min.

Hughes and Saunders (1970) using trout of similar weight and temperature calculated that gill resistance, although variable, did not drop markedly until $\dot{V}_G$ exceeded 300 ml/min. The present study indicates that gill sieve resistance does not show a marked change that can be correlated with $\dot{V}_G$ over the range of 40–160 ml/min. Over much of the physiological range therefore, $\dot{V}_G$ may be adjusted by changes in mean differential pressure alone, that is in the force generated by the respiratory pumps. The configuration of the gills to the water flow, although varying during each breathing cycle, probably does not change with variations in $\dot{V}_G$ over much of the physiological range. Under such conditions, large increases in non-respiratory water spillage (anatomical dead space, Randall 1970) would not occur over the physiological range for $\dot{V}_G$.

Hughes and Shelton (1958) observed changes in gill resistance in the tench at relatively low ventilation volumes. Differences in results between this and Hughes and Shelton's study may be related to a species difference or experimental technique.

There was a weak relationship between area mean buccal or opercular cavity pressures and $\dot{V}_G$ in this study. The relative importance of the buccal and opercular pumps varied between fish and in individual fish with time. Changes in area mean buccal or opercular cavity pressures alone therefore, are not always indicative of changes in water flow over the gills.

The percent utilization of oxygen from water only declined slightly but $\dot{V}_{O_2}$ increased when $\dot{V}_G$ rose from 40 to 120 ml/min. Other studies (Cameron and Davis 1970) indicated that in these experiments the arterial blood was 95–100% saturated with oxygen and venous oxygen content varied little. The increase in $\dot{V}_{O_2}$ with $\dot{V}_G$ must therefore have been largely associated with an increase in blood flow and possibly blood distribution in the gills, maintaining high percent utilization of oxygen in the face of increased velocity and decreased residence time for water in the gills.

Hughes and Saunders (1970) and Marvin and Heath (1968) observed a similar $\dot{V}_{O_2}$ rise in fish with

elevated breathing activity in response to hypoxia. Hughes and Saunders concluded that the elevated oxygen uptake was due to the increased metabolic cost of breathing; however, as pointed out by Cameron and Cech (1970), a variety of factors may contribute to the elevated $\dot{V}_{O_2}$. It is likely that activity and restlessness accounted, to a large extent, for the $\dot{V}_{O_2}$ increase in this study as the higher $\dot{V}_G$'s were recorded after the fish struggled spontaneously or had been disturbed by tapping on the box.

Since we measured pressure across the gills at different $\dot{V}_G$'s we can calculate the external work done in breathing (Alexander 1967; Shelton 1970). Resistance to water flow occurs at the mouth, across the gills, and at the opercular cleft. Pressure differentials across the mouth and opercular cleft are small when these are patent, and work done in moving water through the mouth and out via the gill cleft was calculated and proved to be negligible in this instance. At a $\dot{V}_G$ of 40 ml/min the mean differential pressure across the gills was 0.094 mm Hg (Table 1), or 0.122 cm H_2O. Since a pressure of 1 cm H_2O, equals 1000 dynes/cm^2 and an erg is a dyne-cm, then at a $\dot{V}_G$ of 40 ml/min the work done was (.122 $\times$ 1000)40 = 4880 ergs. This is equivalent to 0.0001125 cal (1 cal = 43.3 $\times$ 10^6 ergs).

Alexander (1967) assumed that the maximum efficiency of the muscles could not exceed 20%. Jones (1971) believed that the efficiency of the respiratory pump was lower (around 3–4%). No one has measured the efficiency of the branchial pump in fish but it is likely low due to the morphology of the respiratory musculature and the way these muscles apply force to the branchial chambers (D. R. Jones personal communication). If we assume that the pump is only 1% efficient, then 0.0001125 $\times$ 100 = .01125 cal are needed to pump 40 ml H_2O /min through the gill chamber. Since 1 ml of O_2 yields about 5 cal (Alexander 1967), then .01125/5 = .00225 ml O_2 is required to support a $\dot{V}_G$ of 40 ml/min. Expressed as a percentage of the total oxygen uptake rate the calculated cost of breathing would be 1.4% of the uptake rate. Table 2 summarizes the oxygen cost of breathing calculated in this way from the data of Table 1, assuming five different efficiencies for the branchial musculature. Clearly the calculated oxygen cost of breathing is very low even if the branchial musculature is highly inefficient. Such a low cost would not be sufficient to account for the elevated oxygen uptake observed when $\dot{V}_G$ increases.

In summary, these experiments indicate that a close relationship between ventilation volume and mean differential pressure across the gill sieve exists. Mean buccal and cleithral pressure appeared poorly related to $\dot{V}_G$. The calculated resistance of the gill sieve appears to change little over the physiological range for $\dot{V}_G$ in rainbow trout. The calculated oxygen cost of breathing based on these experiments was small.

TABLE 2. Oxygen costs of breathing for the fish in Table 1, calculated as percentages of $\dot{V}_{O_2}$ at various efficiencies of the branchial pump for four ventilation volumes.

	Pump Efficiency (%)				
$\dot{V}_G$ – ml/min	1	3	5	10	20
40	1.4	0.47	0.28	0.14	0.07
80	3.86	1.28	0.80	0.39	0.19
100	4.66	1.55	0.93	0.47	0.24
120	6.72	2.23	1.33	0.67	0.34

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Effects of Handling on Oxygen Requirements of American Shad (*Alosa sapidissima*)[1,2]

MARK E. CHITTENDEN JR.[3]

Department of Environmental Sciences
Rutgers, The State University, New Brunswick, N.J., USA

CHITTENDEN, M. E., JR. 1973. Effects of handling on oxygen requirements of American shad (*Alosa sapidissima*). J. Fish. Res. Board Can. 30: 105–110.

Oxygen tolerance was determined 0.25, 3, and 24 hr after introducing young American shad (*Alosa sapidissima*) to washtubs 7, 4, 2, 5, 2, and 2 days after previous disturbance in the holding tank. Dissolved oxygen at equilibrium loss and death varied from 1.1 to 8.0 and 0.9 to 5.7 mg/liter, respectively; and mean oxygen levels at equilibrium loss and death in the 3-hr × 2-day tests were 1.5–3 times higher than in any other handling combination. Present evidence indicates that the effects of handling American shad vary, with the intensity of excitement generated by handling seeming to determine whether the fish lives, dies immediately, or dies later. This phenomenon is probably responsible for difficulty in transporting and marking this species and for conflicting reports on oxygen requirements and salinity and low temperature tolerance. Mean oxygen levels with 99% confidence limits at equilibrium loss and death of unexcited individuals were 1.62 ± 0.13 and 1.28 ± 0.19 mg/liter, respectively. Virtually no individuals lost equilibrium or died until oxygen declined to 2.56 and 1.92 mg/liter, respectively. Nearly all lost equilibrium or died at 0.68 and 0.64 mg/liter, respectively. Field and laboratory evidence suggests that minimum daily oxygen levels of 2.5–3.0 mg/liter permit migration through polluted areas, but 4.0 mg/liter appears necessary in spawning areas.

CHITTENDEN, M. E., JR. 1973. Effects of handling on oxygen requirements of American shad (*Alosa sapidissima*). J. Fish. Res. Board Can. 30: 105–110.

L'auteur a mesuré la tolérance d'oxygène de jeunes aloses savoureuses (*Alosa sapidissima*) 0.25, 3, et 24 h après transfert dans des baquets 7, 4, 2, 5, 2, et 2 jours après manipulation dans le vivier de réserve. Les niveaux d'O_2 auxquels se produisent la perte d'équilibre et la mort varient de 1.1 à 8.0 et de 0.9 à 5.7 mg/litre, respectivement; les niveaux moyens d'O_2 au moment de la perte d'équilibre et de la mort pendant les essais de 3 h × 2 jours sont 1.5 à 3 fois plus élevés qu'au cours de toute autre combinaison de manipulations. Les résultats à date indiquent que les effets de la manipulation de l'alose varient; l'intensité de la surexcitation détermine probablement si le poisson survit, meurt immédiatement ou plus tard. Ce phénomène est sans doute responsable des difficultés de transport et de marquage de cette espèce, ainsi que des rapports contradictoires sur ses besoins en O_2 et de salinité et sur sa basse tolérance thermique.

Les niveaux moyens d'O_2, avec intervalle de confiance à 99%, auxquels se produisent la perte d'équilibre et la mort de sujets non surexcités sont de 1.62 ± 0.13 et 1.28 ± 0.19 mg/litre. Pratiquement aucun sujet ne perd l'équilibre ou ne meurt avant que le niveau d'O_2 baissent à 2.56 et 1.92 mg/litre, respectivement. Presque tous perdent l'équilibre ou meurent à des niveaux de 0.68 et 0.64, respectivement. Les observations sur le terrain et au laboratoire suggèrent qu'un niveau minimum quotidien d'O_2 de 2.5 à 3.0 mg/litre permet le passage à travers les régions polluées, mais qu'un niveau de 4.0 mg/litre est nécessaire dans les régions de fraie.

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[1]Paper in the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, The State University, New Brunswick, N.J.

[2]Based on part of a Ph.D. Thesis, Rutgers University, New Brunswick, N.J., USA.

[3]Present address: Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843.

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THE American shad (*Alosa sapidissima*) formerly was one of the most abundant anadromous fishes in the United States where more than 50 million lb were landed in 1896. In contrast, only 8 million lb were caught in 1960 (Walburg and Nichols 1967). Many workers, including Ellis et al. (1947), Mansueti and Kolb (1953), Talbot (1954), Sykes and Lehman (1957), and Walburg and Nichols (1967) have considered pollution, particularly low dissolved oxygen, a possible factor in the decline of this species. Ellis et al. (1947), Burdick (1954), Tagatz (1961a), and Hoff et al. (1966) have reported oxygen tolerance of this fish; but their results diverge widely, oxygen levels at death varying from about 1 mg/liter (Tagatz 1961a; Hoff et al. 1966) to about 4 or 5 mg/liter (Ellis et al. 1947; Hoff et al. 1966). Hoff et al. suggested that handling of the test specimens greatly influenced oxygen levels at death. Effects of handling were examined in the present studies by measuring oxygen levels at death and equilibrium loss of American shad simultaneously subjected to two handling factors defined below, and some oxygen requirements of this fish are described.

Materials and Methods

The experiment was a 3 × 6 factorial with acclimation period and transferral interval as handling factors whose effects were examined. Acclimation period is defined as the period of time that elapsed between introduction of fish to the test vessels and the beginning of oxygen withdrawal. Levels of this factor were 0.25, 3.25, and 24 hr. Transferral interval is defined as the period of time that elapsed between successive removals of batches of test fish from the holding tank for introduction to the test vessels. Levels of this factor were 7, 4, 2, 5, 2, and 2 days in the sequence of experimental removals. Three test vessels were used on each date when fish were transferred. Acclimation periods were randomly assigned to vessels, and each vessel received three randomly assigned fish. Variation in experimental procedure between vessels was assumed negligible in comparison to the expected effects of handling.

Young American shad, collected in one lot from fresh water of the Delaware River, New York, USA, and transported to the laboratory using procedures described by Chittenden (1971b), were maintained in a holding tank 2.4 m long, 0.9 m wide, and 0.3 m deep with opaque walls and 700 liter capacity. The fish were fed daily a commercial brand of brine shrimp. The tank contained constantly filtered water that was changed fortnightly before tests started and was cleaned daily to remove excess food. No fish died in the holding tank during the 60-day period between collecting the fish and completing the tests.

The test vessels were 57-liter washtubs painted light gray with nontoxic epoxy paint. A floating net was used to trap fish in the holding tank and confine them in a small area. Individuals were removed from the net with a scoop (Chittenden 1971b) and were transferred about 2 m distance to the washtubs. Deoxygenation of aquaria began at 0845–1030 or 1200–1330 hr, and oxygen was removed at a desired rate by bubbling nitrogen gas into the static water at predetermined pressure. Oxygen decreased to values of 3, 2, and 1.5 mg/liter in mean times of 61 (range 56–72), 81 (74–93), and 97 min (87–118), respectively. Preliminary studies showed dissolved oxygen homogeneity within vessels during deoxygenation.

The laboratory lights automatically turned on at 0500 and off at 2100 hr. A 150-w and a 75-w floodlight reflected off the ceiling above the holding tank and washtubs, respectively, minimized excitement of the fish when lighting conditions changed abruptly. The fish were maintained and tested at 16.7–18.3 C in dechlorinated tap water which was continuously aerated except during deoxygenation. The mean total length and weight of the test fish was 83 mm (range 71–100) and 3.6 g (range 2.3–6.3).

Water samples were taken at 15-min intervals and when the last fish died in each test for dissolved oxygen determination by the Winkler method. Behavior was related to the oxygen level by plotting dissolved oxygen against time. Behavior patterns observed in these studies were: death, no visible movement occurred in 15 sec observation, and equilibrium loss, the fish was first noticed on its side or back.

Results

EFFECTS OF HANDLING

Dissolved oxygen varied from 1.1 to 8.0 mg/liter at equilibrium loss and from 0.9 to 5.7 mg/liter at death. Four of 54 fish died prior to oxygen withdrawal. Dummy values were computed for the missing data (Steel and Torrie 1960) to simplify analysis of variance calculations. Interaction between acclimation period and transferral interval was significant at $\alpha = .005$ for both equilibrium loss and death ($F = 8.88$ and 10.31, respectively, each with 9 and 33 df).

Dissolved oxygen at equilibrium loss and death was much higher when a 3-hr acclimation period was combined with a 2-day transferral interval (Fig. 1), explaining the significant interaction. The lowest oxygen level at equilibrium loss and death for the nine fish in that treatment combination was 3.3 and 2.7 mg/liter, respectively; in contrast, the highest value for the 41 fish in all other test conditions was 2.5 and 2.2 mg/liter, respectively. Each fish in the 2-day × 3-hr combination died at a higher oxygen level than that causing equilibrium loss in any other test. The fish that died prior to oxygen withdrawal in the 24-hr × 2-day tests died at about 9 mg/liter 4, 4, 5.5, and sometime between 5.5 and 14.5 hr after transferral. This was about when the 3-hr tests were conducted.

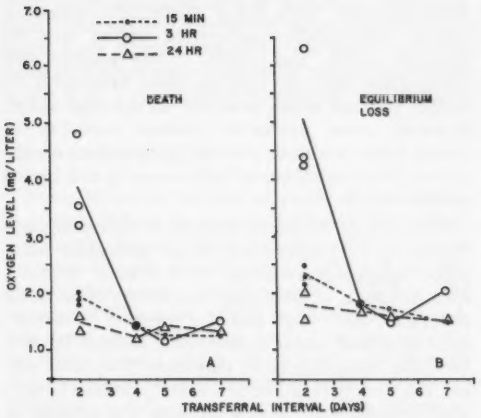

FIG. 1. Effects of transferral interval and acclimation period on oxygen levels at death (A) and at equilibrium loss (B). Dummy values were excluded in calculating treatment combination means presented as data points. For the 2-day transferral interval, lines indicate mean oxygen level for each acclimation period.

Oxygen levels at equilibrium loss and death were similar between transferral intervals of 4, 5, and 7 days, between acclimation periods on those transferral intervals and in the 24-hr acclimation period × 2-day transferral interval combination. Dissolved oxygen at equilibrium loss and death was slightly higher in the 0.25-hr acclimation period × 2-day transferral interval tests than in any other conditions except the 3-hr period × 2-day interval.

Behavior further illustrates the effects of handling American shad in fresh water. The school breaks up just before a seine is landed, and the young swim excitedly about and die within seconds of their initial contact with the net. Fish transferred to aquaria often dash about and almost immediately lose equilibrium, although they gradually calmed down without equilibrium loss in the present studies. In this and other studies, many fish immediately lost equilibrium when trapped in the floating net although capture took only a few seconds; they usually recovered immediately after release in the holding tank and dashed away without apparent delayed effects. Similar equilibrium loss and recovery occurred if the sides of the washtub flexed when carrying fish from the river to the transportation tank.

OXYGEN REQUIREMENTS

The 4- to 7-day transferral interval tests were used to describe oxygen levels at equilibrium loss and death because handling effects seemed minimal. Residuals were examined for outliers (Draper and Smith 1966; Dixon and Massey 1969) to further minimize bias due to handling. One oxygen value at death and at equilibrium loss in the 0.25-hr × 7-day cell and one in the 3-hr × 4-day cell were outliers.

Means, variances and 99% confidence limits of the mean for oxygen levels at death and equilibrium loss are presented below. Outliers and dummy values were excluded from calculations, so the sample size was 25.

Behavior	s^2	$\bar{x}$	Lower confidence limit of $\bar{x}$	Upper confidence limit of $\bar{x}$
Death	.051	1.28	1.15	1.41
Equilibrium loss	.110	1.62	1.44	1.81

Assuming a normal distribution, it is highly probable that the mean oxygen level at death lies within the confidence limits. Confidence interval techniques can be used to estimate the percentage of exposed individuals which will die at a given oxygen level. Table 1 presents predictions based on the observed mean at death and similar predictions at equilibrium loss. A variance suitable for prediction (Steel and Torrie 1960) was used in calculating the confidence limits for individuals. Values of t were obtained from Fisher and Yates (1957). Predictions based upon the upper and lower confidence limits of the mean can be obtained by addition or subtraction of

TABLE 1. Summary of predicted dissolved oxygen levels at death and equilibrium loss of American shad (*Alosa sapidissima*).

	Oxygen Level (*mg/liter*)	
% of Fish	Death	Equilibrium loss
.5	1.92	2.56
1.0	1.85	2.46
5.0	1.67	2.20
10.0	1.58	2.07
20.0	1.48	1.91
30.0	1.40	1.80
40.0	1.34	1.71
50.0	1.28	1.62
60.0	1.22	1.53
70.0	1.16	1.44
80.0	1.08	1.33
90.0	0.98	1.17
95.0	0.89	1.04
99.0	0.71	0.78
99.5	0.64	0.68

0.126 and 0.185 to the oxygen values for death and equilibrium loss, respectively.

The observed mean oxygen level at death was 1.28 mg/liter, and only 0.26 mg/liter separates its confidence limits. Complete mortality would occur by the time the dissolved oxygen declined to 0.51 mg/liter if the lower confidence limit of the mean was used for prediction. Virtually no mortality would be expected until the dissolved oxygen declined to 2.05 mg/liter if the upper confidence limit was used for prediction. The difference in oxygen level between virtually complete and no mortality is about 1.54 mg/liter.

The observed mean oxygen level at equilibrium loss was 1.62 mg/liter with confidence limits of 1.44 and 1.81 mg/liter. Virtually no fish would lose equilibrium until oxygen declined to 2.56 mg/liter if predictions were based upon the observed mean. Predictions for death and equilibrium loss overlap at the lower oxygen levels, and predictions for death take precedence.

The fish repeatedly lost and regained equilibrium when this behavior stage was first noticed. Later they alternated violent, spasmodic bursts of swimming while inverted with quiet periods when they remained inverted on the bottom. The fish ventilated rapidly and with great amplitude when equilibrium loss first occurred, but the rate and amplitude progressively decreased and became more spasmodic until death occurred.

Discussion

Effects of Handling

Many instances have been reported of delayed mortality of fishes after hyperactivity or handling, and this has been associated with lactic acid accumulation in the blood (Black 1958; Beamish 1966; Caillouet 1968). The pattern of dissolved oxygen at equilibrium loss and death in the 2-day transferral interval tests is similar to patterns of blood lactic acid following excitement and death of haddock, *Melanogrammus aeglefinus*, reported by Beamish (1966). He found that blood lactic acid was low before excitement, increased to a peak about 1.5–4 hr later, and then returned to normal after 12 hr. He noted that similar blood lactic acid patterns have been reported for many fishes. Dominy (1971) observed a five-fold increase of blood lactic acid in the alewife, *Alosa pseudoharengus*, after 15 min of chasing. Blood lactic acid, the product of glycolysis to meet energy needs, probably accumulated in the American shad which were excited by handling.

Probable causes of delayed mortality include disturbance of acid–base balance and inadequate oxygenation of blood due to a Bohr effect (Black 1958; Beamish 1966). Sykes (1950) and Chittenden (1971b) successfully transported young only in brackish water, and Hollis (1948) marked fish successfully only by holding them afterwards in Ringer solution which is similar to brackish water. Brackish water minimizes delayed mortality of young American shad, possibly by acting as an ion source which assists blood buffer capacity and lessens a Bohr effect.

Response of American shad to handling suggests Turner's (1971) description of an adrenaline influenced emergency response to a stressor stimulus such as attack, combat, etc. The effects of handling American shad vary, and the intensity of excitement generated seems to determine whether the fish lives, dies immediately, or dies later. With fresh water, the time period about 3–6 hr (possibly longer) after introduction to new aquaria was critical to American shad in the present studies. The much higher dissolved oxygen at equilibrium loss and death in the 3-hr $\times$ 2-day combination indicates that some substance in the fish was depleted after handling. Replenishment sufficient to minimize delayed mortality seemed to take about four days.

The literature on oxygen requirements and salinity and low temperature tolerance of American shad is conflicting. Tagatz (1961b) reported that young were not tolerant of transfer to salt water although Leach (1925), Sykes (1950), and Chittenden (1969) reported extreme tolerance of salinity increase. Tagatz (1961b) found greater tolerance of salinity decrease than I observed. Tagatz (1961b) reported greater sensitivity to temperature decrease than Chittenden (1972). Ellis et al. (1947) reported death of many young before oxygen declined to 4 or 5 mg/liter, indicating much less tolerance of low oxygen than Tagatz (1961a) found. The present studies and those of Hoff et al. (1966) found great variation in oxygen levels at equilibrium loss and death. Handling stress is the probable reason for difficulties in transporting and marking American shad and for apparent sensitivity observed in some experimental work.

Only acclimation period and transferral interval were examined in the present studies, but handling should probably be considered to include all the artificial procedures fish are subjected to during and after capture. Some procedures may not be important stressors, and it may be difficult to identify specific stressors. Proper interpretation of "tolerance" studies requires preliminary evaluation of the effects of experimental procedures and history of handling. This may be especially important with tests begun immediately or shortly after introduction of fish to vessels and with pelagic marine fishes whose blood (Lagler et al. 1962) exhibits a marked

Bohr effect and who behave similar to American shad when in an aquarium or when captured in a seine. Even where precautions are taken, handling stress may affect some individuals as the two outliers in the 4 to 7 day transferral interval tests suggest.

Oxygen Requirements

Mean dissolved oxygen at death of presumably unstressed fish in the present studies (1.28 mg/liter) is similar to Tagatz's (1961a) finding of 50% mortality when oxygen declined to 0.9 and 1.4 mg/liter in brackish water (salinity about 6–11‰). The findings of Ellis et al. (1947) probably reflect handling stress. Oxygen levels at death, however, may not be those causing death when levels continuously decrease; because resistance may permit temporary existence at lethal concentrations. The effects of oxygen reduction rate should be further studied. Dissolved oxygen may fluctuate greatly due to tidal action or to diurnal variation in photosynthesis in a polluted area, and rate of oxygen change and resistance of fish may be important to survival.

Burdick et al. (1954) considered the death point as the oxygen concentration at equilibrium loss. Changes in ventilation during the equilibrium loss stage in the present studies indicate respiratory failure of American shad. Chittenden (1971a) reported respiratory failure of striped bass, *Morone saxatilis*, at equilibrium loss and that oxygen levels at equilibrium loss closely approximate short-term lethal levels and may result in abrupt fish kills.

Tagatz (1961a) maintained young American shad 96 hr without mortality at oxygen levels of 1.8–3.8 mg/liter. Burdick (1954) reported that Talbot (undated) had maintained this fish at "well below" 4 mg/liter with no apparent adverse effects, and Burdick collected normal young in areas of the Hudson River, N.Y., USA having less than 4–5 mg/liter oxygen. Minimum daily dissolved oxygen greater than 2 mg/liter was necessary for adults to migrate past the Philadelphia area of the Delaware River (Chittenden 1969).

There is no sharp distinction between suitable and unsuitable oxygen concentrations, because duration of exposure determines the intensity of the response even at lethal levels. Field and laboratory evidence indicates that minimum daily oxygen levels of about 2.5–3 mg/liter permit existence of most American shad when water temperatures are 15–21 C and this may be sufficient in areas through which American shad just simply migrate rapidly. However, this provides little margin of safety against an abrupt fish kill if oxygen fluctuations occur. In addition, mere survival may not be "reasonably normal" existence. In nature fish must reproduce, be active, feed, grow etc. (Tarzwell 1958; Fry 1960). Whitworth and Bennett (1970) found that eggs did not tolerate oxygen fluctuations from 7.7 to 3.0 mg/liter at 19.8–21 C. Bradford et al. (1968) successfully hatched eggs at 4 mg/liter, but many larvae were "crippled" at lower concentrations. Marcy (1972) found no eggs where dissolved oxygen was less than 5 mg/liter. About 4 mg/liter seems to be the minimum permissable daily oxygen level in spawning areas.

Chittenden (1971a) described a syndrome of reactions to low oxygen that seem to represent a general phenomenon among fishes. Reactions included increasingly violent constant swimming along the aquarium sides, refusal to feed, swimming in bursts of spasmodic activity particularly at the surface, resting with the stomach on the bottom, equilibrium loss and death. American shad reacted that way in the present studies or in earlier ones (Hoff et al. 1966), and also stopped schooling shortly before equilibrium loss. These reactions, some of which began at about 3.5 mg/liter, suggest an attempt by the American shad to adapt to or leave an environment which would be unfavorable in the long run. Doudoroff and Shumway (1967) summarized the sublethal effects of low oxygen and reported that swimming ability, development, and growth may be limited even near saturation values. Repeated exposure to low oxygen as diurnal fluctuations cause can have adverse effects almost as pronounced as continuous exposure (Bouck and Ball 1965). The effects of sublethal dissolved oxygen need further study, but requirements of American shad appear sufficiently high that dissolved oxygen may affect production in some rivers where this species was formerly more abundant. The effect depends, in part, upon the location of pollution in relation to production areas. Pollution upstream of the chief production areas may be least critical because the young need not pass through it, and mild eutrophication may actually support greater biomass. Pollution downstream of production areas may be very critical because the young migrate to sea.

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OBITUARY

Wallace Allison MacCallum

1918-1972

Mr W. A. (Wally) MacCallum, head of the Technological Division, Fisheries Research Board of Canada, Biological Station, St. John's died at the St. John's General Hospital on August 28, 1972, after a long illness. He is survived by his wife and three sons.

Mr MacCallum was born in Amherst, Nova Scotia, in 1918. He was a graduate of Mount Allison University (B.Sc. Honours) in 1939, the Nova Scotia Technical College (degree in Mechanical Engineering) in 1941, and Dalhousie University (M.Sc. in Theoretical Physics) in 1948. He served as an Officer in the Canadian Army (R.C.E.M.E.) from 1944 to 1946. He joined the Fisheries Research Board of Canada, Atlantic Fisheries Experimental Station, at Halifax in 1946.

Prior to his army service he worked as an engineer with commercial firms in Pictou, Nova Scotia, and Latuque, Quebec.

While in Halifax, Mr. MacCallum developed methods of handling fish catches on board vessels and studied fishing vessel hold design and refrigeration methods. The results of this work had wide application both in Canada and other fishing nations.

In 1953 he was seconded to the Food and Agriculture Organization for a short period to enable him to go to Yugoslavia to assist with the collection, transportation, storage, and refrigeration of fish in that country. Subsequently, he studied problems of mechanical washing of salted fish and was granted a patent for the design of a fish washing machine that received wide recognition by the fishing industry.

In 1960 Mr MacCallum transferred to St. John's to take charge of the Newfoundland Technological Unit of the Fisheries Research Board where he continued work on fish handling, processing, and preservation with specific reference to problems of the Newfoundland industry, including methods to improve the quality of cod products from the inshore fisheries and engineering and economic investigations relating to thawing and processing of fish frozen at sea.

During his career Mr MacCallum was an active member in various professional organizations. Nationally and internationally known for his work on fish handling and preservation and the production of many scientific and engineering papers on these subjects, he is especially noted for his sound engineering research and practical application of results to industry.

H. E. Power
A. M. Fleming

NOTES

Seasonal and Local Variations in Odd-Chain Fatty Acid Levels in Nova Scotian Rainbow Smelt (*Osmerus mordax*)

R. F. ADDISON

Fisheries Research Board of Canada
Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S.

R. G. ACKMAN AND J. HINGLEY

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ADDISON, R. F., R. G. ACKMAN, AND J. HINGLEY. 1973. Seasonal and local variations in odd-chain fatty acid levels in Nova Scotian rainbow smelt (*Osmerus mordax*). J. Fish. Res. Board Can. 30: 113–115.

Odd-chain fatty acid (OCFA) levels in rainbow smelt (*Osmerus mordax*) from Jeddore Harbour, N.S., showed a pronounced seasonal variation during the period from spring 1969 to fall 1970, reaching a maximum of 20% total fatty acids in the January–March period, and falling to a minimum of approximately 2% total fatty acids in summer and fall. The variation appeared to be general, and occurred in all OCFAs. Analysis of smelt stomach contents suggested that OCFAs originated in the diet. Smelt caught in inlets adjacent to Jeddore Harbour showed no unusual accumulation of OCFAs in January, 1970.

ADDISON, R. F., R. G. ACKMAN, AND J. HINGLEY. 1973. Seasonal and local variations in odd-chain fatty acid levels in Nova Scotian rainbow smelt (*Osmerus mordax*). J. Fish. Res. Board Can. 30: 113–115.

Le niveau des acides gras à chaînes impaires (OFCA) chez des éperlans d'Amérique (*Osmerus mordax*) provenant de Jeddore Harbour, N.-E., ont subi des variations saisonnières marquées au cours de la période allant du printemps 1969 à l'automne 1970. Il atteignait un maximum de 20% des acides gras totaux au cours de la période de janvier à mars, et baissait à environ 2% en été et en automne. Ces variations semblent générales et se produisent avec tous les OCFAs. L'analyse du contenu stomacal des éperlans nous porte à croire que les OCFAs ont leur source dans la nourriture des poissons. Les éperlans capturés dans les anses avoisinnant Jeddore Harbour ne démontrèrent aucune accumulation anormale d'OCFA en janvier 1970.

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WE have described elsewhere the unusual occurrence of large amounts of odd-chain fatty acids (OCFAs) in the lipids of rainbow smelt (*Osmerus mordax*) from Jeddore Harbour, N.S. (Addison and Ackman 1970). The data suggested that there might be a seasonal variation of OCFA levels in Jeddore smelt, which could be related to the migratory or feeding habits of the fish (MacKenzie 1946). This note describes the results of a study of seasonal variation in OCFA levels on Jeddore smelt from the spring of 1969 to the summer of 1970. Spot analyses of OCFA levels in smelt from adjacent inlets, and of OCFA levels in Jeddore smelt stomach contents are included in this report.

Experimental — Smelt for the seasonal variation study in Jeddore Harbour were caught by gillnetting at 2-week intervals throughout the period May 1969–August 1970. Every third catch was sorted by sex, and fatty acid analyses were carried out. In some cases particularly during summer and autumn 1969, when insufficient fish were available for analysis from one catch, the catch before or after it was analyzed instead.

Smelt from areas other than Jeddore were caught in winter, 1970, by fishing through the ice.

Analytical procedures were essentially the same as described previously (Addison et al. 1968; Addison and

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Ackman 1970). In some cases, fatty acid methyl ester analyses were carried out on a Packard 7400 instrument fitted with 6-ft × ⅛-inch inside diameter coiled glass columns packed with 3% EGSS-X on 100–120 mesh Gas-chrom Q, in addition to the equipment used previously.

Results and discussion — The OCFAs observed in the Jeddore Harbour smelt were those found previously, i.e. mostly 15:0[1], 17:1, and 19:1 with small amounts of 15:1, 17:0, 17:2, 19:0, 19:4, and 21:5, although the latter group was not detected with confidence in samples with low overall OCFA levels. OCFA levels varied widely, reaching a distinct peak at around 20% of total fatty acids in the January–March period, and falling off to more usual levels (1–2% total fatty acids) in the summer and autumn. The increase appeared to be a general one, applying to all OCFAs. The results are illustrated in Fig. 1. There was a parallel decrease in the proportion of 16:0 and 22:6ω3, from approximately 20 to 15% and from approximately 25 to 15% total fatty acids, respectively. The proportions of 18:1 ω9 and 20:5 ω3 were virtually unchanged at approximately 20 and 14% total fatty acids, respectively. Calculated iodine values declined from 215 to 185 with the increase in OCFA levels. These fatty acid changes would be consistent with increasing triglyceride levels (Addison et al. 1968), and the increase in total muscle lipid (from 0.6% to 2.6% wet weight) in the January–March, 1970 period supports this interpretation.

In an attempt to identify the source of OCFAs in smelt tissue, OCFA levels were measured in stomach contents of Jeddore smelt on two occasions during the winter–spring 1970 peak. Results are shown in Table 1. Total stomach contents on January 20 (in which shrimps and an amphipod were recognisable) were rich in OCFAs. A second batch of stomach contents (March 3) was divided somewhat arbitrarily into two fractions, consisting of (a) large particles (shrimp, polychaete setae, and miscellaneous crustacean parts), and (b) presumed semi-digested matter resembling brown mud, and probably algal or detrital components. Of these, the fraction containing the digested material contained considerably more OCFAs than did that consisting of larger particles (Table 1). In all stomach contents, the relative amounts of 15:0, 17:1, 19:1 were roughly similar, and similar to that found in smelt tissue. Although it is realized that smelt stomach may contain endogenous lipid similar in composition to that of adipose tissue, these figures suggest that the OCFAs of smelt adipose tissue are, at least to some extent, of dietary origin. The high level of 20:5 ω3 suggests a predominantly crustacean origin for these stomach contents (Addison et al. 1972).

The only other species of fish known to contain unusually high proportions of odd-chain fatty acids is striped mullet *Mugil cephalus*. Those taken in the Gulf of Mexico show considerable variation in detail probably with season. Obvious odd-chain

[1]Notation to indicate chain length (no. of C atoms): no. of double bonds and position relative to terminal methyl group.

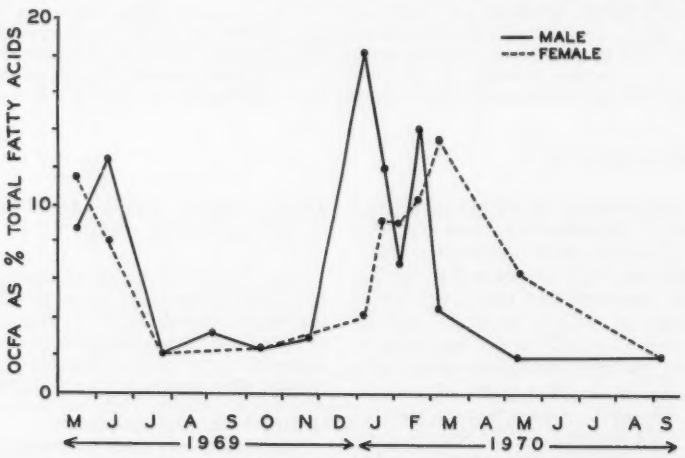

FIG. 1. Seasonal variation in OCFA levels (as percentage of total fatty acids in muscle tissue) in Jeddore Harbour male and female smelt, May 1969–September 1970.

TABLE 1. Fatty acid composition (wt. % of fatty acids C_{12}–C_{24}) of stomach contents of Jeddore Harbour smelt.

Date of sample	Jan. 20, 1970 (*Total*)	Mar. 3, 1970 Large particles	Mar. 3, 1970 Semidigested matter
Fatty acid[a]			
12:0	tr[b]	0.1	0.2
13:0	tr	tr	tr
14:0	2.7	2.7	2.7
14:1	tr	0.1	0.3
15:0	4.1	1.2	2.6
15:1	1.1	tr	0.4
16:0	17.1	18.6	16.6
16:1	9.9	10.1	12.2
16:2 ω7	0.4	tr	0.4
16:3 ω4	0.2	0.3	0.5
16:4 ω1	0.2	0.1	0.5
17:0	1.0	0.6	0.5
17:1	7.0	1.4	5.1
18:0	2.5	3.4	2.5
18:1	19.0	16.6	18.2
18:2 ω6	0.9	1.1	1.0
18:3 ω3	0.4	0.5	0.3
18:4 ω3	0.6	0.8	1.1
19:1	1.7	0.6	1.2
19:4 ω5	1.6	0.3	0.9
20:1	2.0	6.4	1.0
20:4 ω6	3.2	2.3	2.5
20:4 ω3	0.1	0.2	0.6
20:5 ω3	14.2	16.5	18.9
22:1	tr	6.0	tr
22:5 ω3	0.8	1.2	0.9
22:6 ω3	9.2	6.1	8.7
ΣOCFA	16.5	4.1	10.7

[a]Notation for no. of C atoms: no. of double bonds and position of last double bond relative to Me group.
[b]tr: trace.

components range from 3.3% 15:0 and 2.3% 17:1 (Iyengar and Schlenk 1967) to 11.2% 15:0 and 4.6% 17:1 (Sen and Schlenk 1964), but to our knowledge the origin of the odd-chain fatty acids in these fish also remain enigmatic.

During the winter–spring 1970 peak in OCFA levels in Jeddore smelt, smelt from other inlets in the N.S. eastern shore (caught on January 6, 1970) were analyzed. Results are shown graphically in Fig. 2. No fish were available from the nearby Petpeswick or Chezzetcook inlets, due to lack of commercial interest, but smelt from Musquodoboit Harbour and Porter's Lake were essentially normal in OCFA levels. Smelt collected from three geographically remote maritime locations in February and March 1969 also had normal OCFA levels (Addison and Ackman 1970). The present findings

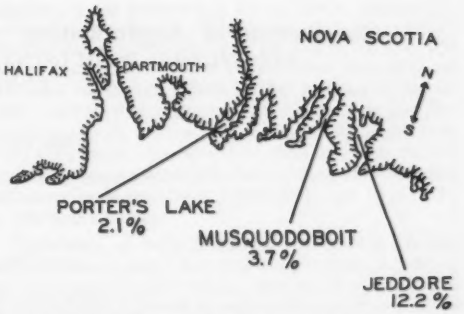

FIG. 2. Map of Nova Scotian eastern shore showing total OCFA levels (as percentage of total fatty acids in muscle tissue) in smelt taken from Porter's Lake, Musquodoboit Harbour, and Jeddore Harbour, N.S., on January 6, 1970.

rule out the possibility of there being a discrete population of smelt peculiar to the Nova Scotian shelf, and in fact, electrophoretic studies have failed to show differences between various Maritime stocks of smelt examined for OCFAs. (P. Odense personal communication).

We conclude from this work that the OCFAs in Jeddore Harbour smelt arise from a dietary source and that the source is either absent from adjacent Eastern Shore inlets or does not form part of the diet of smelt taken in the adjacent inlets. Recent work (Ackman and Paradis, in preparation) suggests that the source of OCFAs in these smelts is the amphipod *Pontoporeia femorata*. Whether this organism synthesizes OCFAs de novo or accumulates them from lower trophic levels remains to be established.

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Production of Agglutinating Antibodies in the Channel Catfish (*Ictalurus punctatus*) Against *Chondrococcus columnaris*[1]

JOHN H. SCHACHTE, JR.

Department of Fisheries and Allied Aquacultures
Alabama Agricultural Experiment Station
Auburn University, Auburn, Ala. 36830, USA

AND EMILIO C. MORA

Department of Poultry Science
Alabama Agricultural Experiment Station
Auburn University, Auburn, Ala. 36830, USA

SCHACHTE, J. H., JR., AND E. C. MORA. 1973. Production of agglutinating antibodies in the channel catfish (*Ictalurus punctatus*) against *Chondrococcus columnaris*. J. Fish. Res. Board Can. 30: 116–118.

Fingerling channel catfish (*Ictalurus punctatus*) were injected subcutaneously and intramuscularly with an injectable *Chondrococcus columnaris* antigen to test for the production of agglutinating antibodies. The test fish of both routes of injection had a mean titer of 1:4337. No significant differences in titers between the two routes of injection were determined.

SCHACHTE, J. H., JR., AND E. C. MORA. 1973. Production of agglutinating antibodies in the channel catfish (*Ictalurus punctatus*) against *Chondrococcus columnaris*. J. Fish. Res. Board Can. 30: 116–118.

Dans le but de vérifier la production d'anticorps agglutinants, nous avons exposé des alevins de barbue (*Ictalurus punctatus*) à un antigène injectable de *Chondrococcus columnaris* par inoculations sous-cutanées et intramusculaires. Les deux voies d'inoculation ont donné chez les poissons expérimentaux un titre moyen de 1:4337. Nous n'avons pas détecté de différence significative de titre entre les deux voies d'injection.

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RECENT studies by Post (1963), Fujihara et al. (1964), Krantz et al. (1963), and Fujihara (1970) demonstrated the presence of agglutinating antibodies against bacterial pathogens in coldwater fishes. The antigen–antibody relation of warmwater cultured fish and specific bacterial pathogens have received little attention. This study was designed to test the antigenicity of *Chondrococcus columnaris* in the channel catfish (*Ictalurus punctatus*) and to measure the resultant production of agglutinating antibodies. Two routes of injection were compared to determine if any difference in titer could be expected from an intramuscular versus a subcutaneous injection of the antigen.

Materials and methods — The isolate of *C. columnaris* used in this study was obtained from a natural epizootic of columnaris disease in channel catfish fingerlings in a running water trough maintained at 28 C in the laboratory. A liquid cytophaga medium consisting of 0.4% Bactotryptone, 3% Bacto-yeast infusion, and 1% "Tween 80" (Anacker and Ordal 1955) with a *p*H of 7.2 was used to grow bacterial cells for antigen preparation. The medium was inoculated and maintained in a shaker incubator at 25 C for 72 hr, then the bacterial cells were centrifuged at 13,000 $\times g$ for 15 min in a refrigerated centrifuge. The cells were washed five times in .85% saline, then suspended in saline at a concentration that when diluted 1:20 had an OD of 0.15 at 600 nm. Six-week-old White Leghorn chicks were injected intravenously in the wing vein with 0.1 cm^3 of the live *C. columnaris* suspension every other day for a total of three injections. One week following the third injection, a booster of 0.1 cm^3 of antigen was given and serum was obtained 3 weeks postinoculation. The chick antiserum was titered using a stained *C. columnaris* antigen and the slide agglutination technique (McEwen 1950). The chick antiserum was used as the known positive control.

Selection and care of experimental fish — Sixty-five 8-month-old fingerling channel catfish produced at the

[1]This research supported in part by the Southeastern Cooperative Fish Parasite and Disease Project.

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Fisheries Research Unit of the Auburn University Agricultural Experiment Station were randomly selected, weighed, and measured. The fish ranged from 20 to 40 g in weight and from 12.5 to 17.5 cm in length. Five fish were used to check for naturally occurring antibodies against *C. columnaris* prior to stocking the aquaria. The fish were placed randomly, six in each of ten 40-liter aquaria. The aquaria were aerated and clean; municipal water, filtered for chlorine removal, was maintained at 28 C. The *p*H of the water ranged from 6.9 to 7.1 throughout the experimental period.

Within each aquarium one fish was identified as a noninoculated control by a pectoral fin clip. The remaining five fish were designated as test fish. The 10 aquaria were divided into two groups of five. Fish in one group received intramuscular injections of the antigen and the second group received subcutaneous injections.

Preparation of the injectable antigen — Two 125-ml flasks of liquid cytophaga media were inoculated with *C. columnaris* and incubated at 25 C for 72 hr in a shaker incubator. The cells were packed by centrifugation at 20,000 × *g*. The packed cells were suspended in a sterile 0.85% NaCl solution, heat inactivated in a constant temperature water bath at 58 C for 30 min, and processed according to the patented bacterin technique of Mora (1967). The final concentration of the antigen was adjusted to 0.75 OD at 600 nm.

Inoculation of fish — Two routes of injection were used. One group of 25 fish received intramuscular injections while the second group of 25 fish received subcutaneous injections. Prior to inoculation, all fish in each aquarium were anesthetized in a solution of 2 ppm quinaldine. Each test fish was inoculated with 0.1 cm^3 of the *C. columnaris* antigen immediately posterior to the dorsal fin by the selected route. Three injections were given at 48-hr intervals. One week following the third injection a 0.1 cm^3 booster was administered.

Collection of blood samples — Blood samples were obtained beginning one week following the booster injection. It was necessary to kill the fish to obtain sufficient serum to conduct agglutination tests. The control and test fish were anesthetized, the caudal fin removed at the peduncle, and 0.01–0.5 ml of blood was collected in 75-mm hematocrit capillary tubes and centrifuged at 2000 × *g* for 10 min. The tubes were scored at the cell–serum interfaces, broken, and the serum containing portions were sealed. The sera were identified according to fish source and stored at –20 C for a period of 1–5 months before antibody titers were determined.

Preparation of stained antigen — A stained antigen was prepared in order to obtain sharp contrast in reading the slide agglutination results. One hundred twenty-five milliliters of a 72-hr-old bacterial cell culture in Ordal's liquid cytophaga medium were packed by centrifugation, washed, standardized, and heat inactivated identically as cells used to prepare the injectable antigen. Fifty milligrams of toluidine blue per 25 ml of the original bacterial suspension were added. The stained suspension was refrigerated 18 hr at 4 C, then washed in 0.85% saline five times at 6500 × *g* for 10 min to remove the excess dye. The cells were suspended in 5 ml of 0.85% saline for use in the agglutination test.

Agglutination and titer determination procedures — All reactions considered positive were evaluated with a 2-min time limit. Agglutination reactions were determined at a magnification of 15×. A sheet of frosted acetate was placed on the stage beneath the sample to facilitate the light diffusion through the suspension.

Twofold serial dilutions of the serum were made and one drop of each dilution was mixed with an equal volume of the stained antigen on slides. The slides were rotated to ensure mixing.

Results and discussion — Antiserum collected from chicks had average titers of 1:160 using the stained antigen-slide agglutination technique.

The five preinoculation fish serum samples obtained prior to the beginning of the experiment had no detectable agglutinin antibodies against *C.*

TABLE 1. Serum titers of channel catfish (*Ictalurus punctatus*) inoculated with *Chondrococcus columnaris* antigen using two routes of injection.

	Intramuscular Injection					Subcutaneous Injection				
Aquarium:	1	2	7	9	10	3	4	5	6	8
Fish										
Control	0	0	0	1:160	0	0	0	0	0	0
1	1:4800	0[a]	1:4800	1:4800	0[a]	1:2400	1:2400	1:5120	1:320	1:3200
2	1:1200	0[a]	1:4800	1:2400	0[a]	1:3200	1:400	1:6400	1:4800	1:9600
3	1:9600	0[a]	1:4800	1:4800	1:4800	1:4800	1:2400	1:6400	1:4800	1:9600
4	1:9600	0[a]	1:4800	1:2400	1:2400	1:4800	1:4800	1:6400	1:320	1:4800
5	1:1200	1:4800	1:2400	1:4800	1:4800	0[a]	0[a]	1:6400	1:2400	1:2400

[a]These fish died 3 days after the first injection.

columnaris. At the completion of the experiment, nine of 10 control fish had no detectable titers. One control fish that was accidentally inoculated once with 0.1 cm^3 of the antigen had a titer of 1:160.

The 42 survivors in the experiment had agglutinin titers ranging from 1:320 to 1:9600 (Table 1). Eight fish that received only 0.1 cm^3 of the antigen died at 3 days postinoculation. Three of these fish jumped from the aquaria during the night and the remaining five died of causes unknown. All titers in the test fish were induced within a 3-week period of inoculation with a total of 0.4 cm^3 of the antigen. An analysis of variance (Steele and Torrie 1960) showed no significant differences ($P>0.05$) between the two routes of injection.

The response of the catfish (Table 1) confirms the antigenicity of the organism as well as a marked agglutinin titer in the host fish. The response of the channel catfish is similar to that of the rainbow trout against *C. columnaris* as demonstrated by Fujihara et al. (1964). In the trout the maximum titer obtained was 1:5120, which is similar to the results obtained in this experiment. An average titer of 1:4337 and a maximum titer of 1:9600 developed after 3 weeks postinoculation as compared with 12 weeks in the trout. At the end of 3 weeks trout had a titer of 1:160. The comparison of these data seems to emphasize the role of ambient temperature in antibody production in cold blooded animals (Bisset 1948). This should be taken into consideration because the fish discussed here represent both coldwater and warmwater forms. The possibility that physiological differences are present in the two species, with respect to antibody production, can not be discounted. The lack of significant differences in channel catfish antibody titer against *C. columnaris* based on route of injection closely parallels previous results obtained with rainbow trout (Fujihara et al. 1964). These data indicate that little if any difference in titer can be expected as a result of varying the route of injection in fish. This assumption, however, will have to be further substantiated with other species of fishes as well as different antigens.

Rio and Recco (1971) noted loss of precipitation titer in goldfish (*Carassius auratus*) serum after only 4 days at below freezing temperatures. At the end of 14 days all goldfish serum samples had negative precipitin titers against human serum. In the present study antibody titers were measured during an interval of 1–5 months, during which time the serum was stored at –20 C. Rio and Recco (1971) pointed out that there could be species differences in the stability of antibodies in fish serum.

The data obtained in this study suggest that the possibility exists for the immunization of fish; however, further work must be carried out to establish practical means of large scale immunization.

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Hydrophone and Bow-Mount for Tracking Fish by Ultrasonic Telemetry

A. B. STASKO AND S. M. POLAR

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

STASKO, A. B., AND S. M. POLAR. 1973. Hydrophone and bow-mount for tracking fish by ultrasonic telemetry. J. Fish. Res. Board Can. 30: 119–121.

An inexpensive, do-it-yourself hydrophone for tracking aquatic animals is described. It can be used with or without a preamplifier, on small or large boats. A new mounting arrangement used on the bow of a 25-m tracking vessel is discussed. It was used successfully in coastal waters at cruising speeds up to 9 knots (5 m/sec) and at slow speeds during rough weather in 30-knot (15.3 m/sec) winds.

STASKO, A. B., AND S. M. POLAR. 1973. Hydrophone and bow-mount for tracking fish by ultrasonic telemetry. J. Fish. Res. Board Can. 30: 119–121.

Les auteurs décrivent un hydrophone peu dispendieux, qu'on peut fabriquer soi-même, pour dépister les animaux aquatiques. L'appareil peut être utilisé avec ou sans préamplificateur, à bord de petits et de grands navires. On examine une méthode nouvelle de montage de l'appareil sur la proue d'un navire de 25 m. Les auteurs l'on utilisé avec succès dans les eaux côtières, à des vitesses de croisière allant jusqu'à 9 noeuds (5 m/sec) et à de faibles vitesses, par mauvais temps et vents de 30 noeuds (15.3 m/sec).

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TRACKING of fish and other underwater animals fitted with ultrasonic transmitters is becoming a widely used technique in fisheries research (Stasko 1971). Hydrophones for such work have often been adapted from echo sounders (e.g. Hasler et al. 1969; Johnson 1960) or built for a specific project with inadequate descriptions for duplicating them (e.g. Carey et al. 1971). Directional, low-frequency hydrophones suitable for tracking fish are also available commercially at a cost of several hundred dollars, with some models costing as much as $2000. An inexpensive, do-it-yourself hydrophone, which can be built for less than $40 in material costs, is described here.[1]

The hydrophone (Fig. 1) consists of a reflecting cone (9, 10), a piezoelectric transducer element (G), and a flange (4). The 90° cone is made of noncorrosive metal sheet cut to a pattern and bent to shape, with a sound-reflecting neoprene rubber lining on the inner surface of the cone. The transducer elements are lead-titanate zirconate piezoelectric cylinders. Two such elements are mounted on a threaded metal rod (1) with insulating spacers at both ends and in the middle (D, E, F). Electrically the transducers are connected in parallel. The transducer assembly is held in position inside the cone by a threaded metal or plastic conical nut (5) on the inside and a pipe flange with a nut (4,2) on the outside of the cone.

Sensitivity of this hydrophone at the commonly used ultrasonic tracking frequencies of 50 and 74 kHz is comparable to the most frequently used commercial tracking hydrophone in North America. On the basis of acoustic-tank tests made on two of our hydrophones, the sensitivity is nearly uniform at about –90 db/v/μbar from 20 to 60 kHz, with increased sensitivity of –85 db at 70 kHz, –87 db at 80 kHz and –83 db at 100 kHz. Beam width at the 3 db points is 20° at 30 kHz, and 10° at 50 and 75 kHz.

The hydrophone can be mounted at the end of a hand-held stick or a rotating pipe fixed to the gunwale of small boats. Although this type of hydrophone can withstand speeds of several knots through the water, provision should be made for tilting the shaft out of the water when traveling fast. Such an arrangement has been used on shallow-draught boats up to 15 m long but is unsuitable for larger vessels.

In the one published study where a larger vessel (about 60 m) was used, the hydrophone was part of a complex through-the-hull sonar system (Yuen 1970).

In 1971 we successfully used our own cone-shaped hydrophone on the 25-m long M/V *Harengus*. To do this we built a 7-m long, faired hydrophone shaft mounted over the bow of the boat. A basically similar arrangement, but differently constructed and without a hydrophone housing, has been used on a 19-m boat (K. Lawson personal communication).

[1]Parts list and construction details available on request from S. M. Polar.

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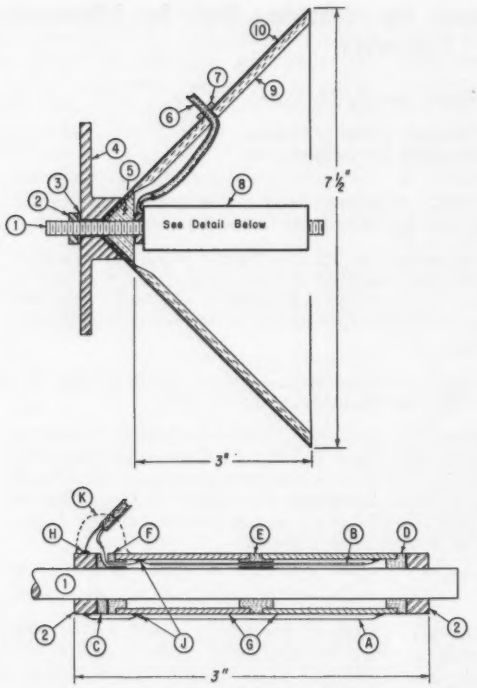


Material Specifications

1, ¼" Threaded shaft
2, ¼–20 Nut
3, Lock washer
4, Deck flange, 1" galvan.
5, Conical nut (custom)
6, Coaxial cable, RG58A/U
7, Rubber grommet, ¼"
8, Epoxy-formed coating
9, Neoprene rubber, ¼"
10, Conical reflector, 90°, 24 awg

A, Outside wire #28
B, Inside wire #28 insulated
C, Insulating washer, notched
D, Insulating spacer, front
E, Insulating spacer, center
F, Insulating spacer, rear
G, Piezoelectric cylinder
H, Epoxy solder joint
J, Silver solder joint
K, Epoxy

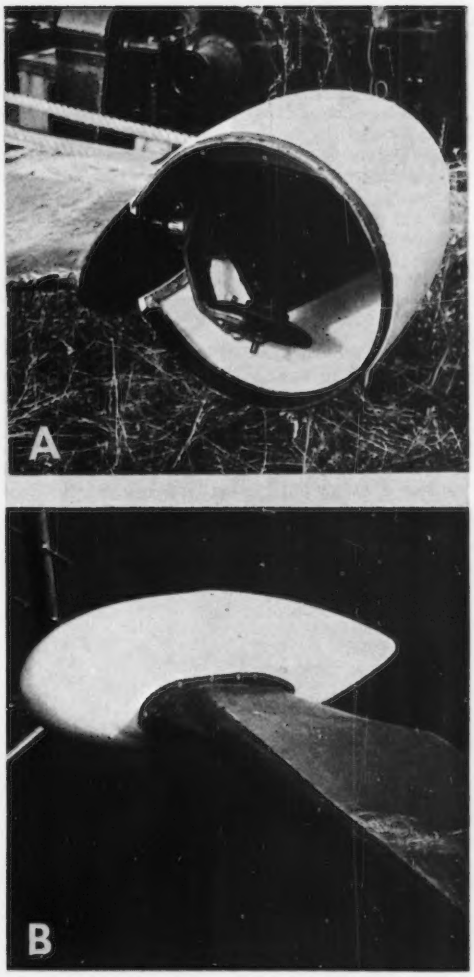
FIG. 1. Conical tracking hydrophone with detail of transducer arrangement.

Our hydrophone mounting arrangement is shown in Fig. 2. The faired shaft was hung on a rigid vertical support post by means of pintels and gudgeons. This attachment allowed the shaft to turn into the stream as the boat rolled or turned sharply, thus always presenting the narrow cross section of both faired shaft and hydrophone housing to the movement of water past them. The hydrophone was attached to the lower end of a cylindrical shaft mounted inside the faired shaft. This inner shaft with hydrophone could be rotated while the outer shaft swung independently according to the forces of the water against it.

The torpedo-shaped housing (Custom Plastics, Vancouver, B.C., Canada) was made in two halves of ABS plastic 2 mm thick (Fig. 2a, 2b) with several holes drilled in the rear half to allow rapid filling with water when immersed, and rapid emptying when removed from the water. Size and spacing of holes is not critical. Presence of the housing had no effect on the hydrophone output (resolution of test equipment was $\pm 5\%$) when the processed signals from a transmitter immersed 40 m distant were measured on an oscilloscope.

Attachment of the housing to the faired outer shaft was by a flat metal plate screwed to the housing and welded to the shaft. Auto-body filler was used to streamline the transition to the faired pipe. The outer shaft was 60-mm outside diameter pipe. Its fairing (thickness ratio = 0.2) was made from two flat steel plates 2 mm thick welded to the pipe and to each other at the trailing edge. The short-chord fairing above the lower gudgeon was used simply to increase strength against bending. At both ends of the outer shaft a brass bushing supported the inner shaft. A thrust bearing near the top of the 34-mm outside diameter inner pipe prevented it from sliding down while a clamp at the lower end prevented it from sliding up. At the very top end of the inner shaft a bicycle sprocket and chain, connected by steering cable to a similar sprocket and chain in the wheelhouse, were used to manually rotate the hydrophone shaft from the wheelhouse.

The whole hydrophone assembly was attached by pintels and gudgeons to a vertical 90-mm outside diameter pipe. This support post was rigidly bolted at the top to a solid channel-iron frame attached to the foredeck and, at the lower end, to two braces (48-mm outside diameter pipe, 2.9 m long). These braces must be strong and spaced far enough apart at the front end (450 mm in our mount, but could be reduced with only slight modification in design) to allow the faired shaft to swing 90° in either direction. For ease of attaching the movable hydrophone mount, the lower pintel was made longer than the upper one and ladder-like rungs were welded



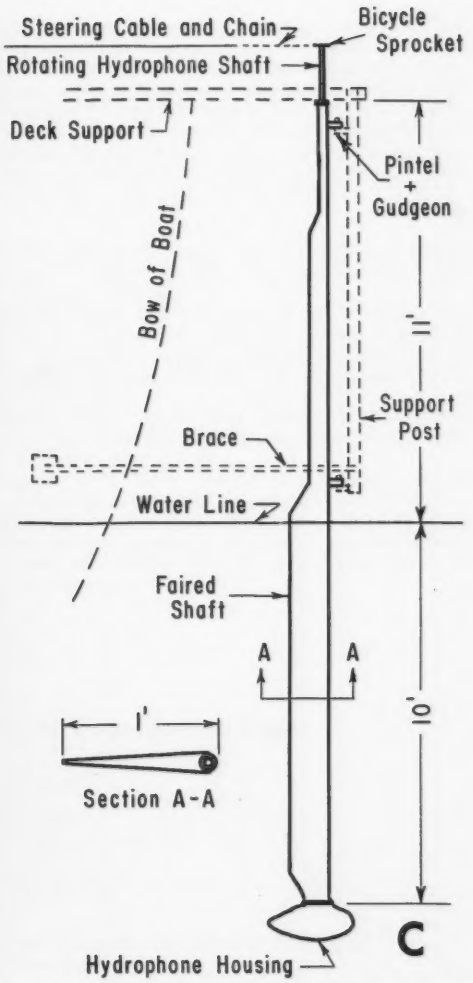


FIG. 2. Hydrophone mounting arrangement for a large boat: (A) conical hydrophone inside protective housing; (B) streamlined housing at end of faired shaft; and (C) drawing of entire hydrophone mount attached to bow of tracking vessel.

Stasko and Polar — J. Fish. Res. Bd. Canada

to the rigid support post so that a man could climb down and guide the shaft when lowering the hydrophone mount into position.

Electrically the hydrophone was connected via coaxial cable through the inner shaft to a low-noise preamplifier (Ithaco, model 144F) on deck near the bow. A 1 Mohm resistor was shunted across the input to eliminate low frequency signals which tended to block the preamplifier. A 0.33-μf capacitor was put in series with the output line for DC blocking and high-pass filtering. The preamplifier, in addition to giving 40 db amplification, converted the output to low impedance required by a low input impedance ultrasonic receiver (50 to 100 ohms) and useful with a medium impedance ultrasonic receiver (68 kohms). There was noticeable improvement in signal-to-noise ratio for weak signals, resulting in actual increase of signal range (estimated at 10–20% compared with the same hydrophone without preamplifier). Some further reduction in electrical noise might be achieved by placing the preamplifier next to the hydrophone.

The hydrophone mount was used successfully to track Atlantic salmon in the Gulf of St. Lawrence some 30 km offshore from the mouth of the Miramichi River. We were able to travel at full speed (about 9 knots) in calm weather and to track (average speed of fish was 1.0 knots) in winds up to 30 knots. Ambient noise from waves, a serious problem for side-mounted hydrophones, increased with increase in wind speed but was not serious presumably because the hydrophone was ahead of the bow. Increase in ship speed produced very little acoustic noise, except for propeller noise when hydrophone was pointed astern. We could hear signals from a sonic-tagged salmon while traveling at full speed.

Acknowledgments — The hydrophone described here evolved from earlier models made by J. Johnson (National Marine Fisheries Service, Seattle) and A. D. Hasler's team at the University of Wisconsin. Acoustic-tank tests were done by the Defence Research Establishment Atlantic in Halifax, N.S. The bow-mount was designed through the pooled advice of K. Lawson, P. Ryan, J. Beckett, T. Foulkes, J. Carrothers, and H. Small. Figures were prepared by P. W. G. McMullon.

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Winter Surface Currents Around Cape Breton Island

R. GRANT INGRAM

Marine Sciences Centre
McGill University, Montreal, Que.

INGRAM, R. G. 1973. Winter surface currents around Cape Breton Island. J. Fish. Res. Board Can. 30: 121–123.

The winter surface circulation around Cape Breton Island was studied by tracking ice fields. The resulting streamlines compare favorably with prior work.

INGRAM, R. G. 1973. Winter surface currents around Cape Breton Island. J. Fish. Res. Board Can. 30: 121–123.

La circulation de surface autour de l'île du Cap-Breton durant l'hiver a été obtenue en suivant le mouvement des champs de glace. Les lignes de courant se comparent favorablement aux résultats de travaux antérieurs.

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IN the past, a variety of methods have been employed to map the surface circulation in large bodies of water. Of these, drift bottles and instrumented buoys have been the most frequently applied techniques. However, free-drifting tracer methods are obviously of limited value in most Canadian waters during winter, when a full or partial ice-cover is established. Since a knowledge of the winter circulation and its subsequent influence on the drift of sea ice is of major importance to shipping and other studies, alternate methods are necessary.

One area of specific interest is that surrounding Cape Breton Island, where the winter circulation is poorly documented. In one of the few available references, Bumpus and Lauzier (1965) showed only two drift bottle results in this area during the winter months. Recently, El-Sabh and Johannessen (1972) have published a winter circulation pattern in the central area of Cabot Strait, incorporating a number of hydrographic observations from different years. Thus, previous methods have met with limited success, either in extent of coverage or feasibility of instrument deployment.

An alternate method of predicting winter circulation is herein discussed, one which has not been reported previously in the literature. This technique consists of tracking ice floe fields, assuming that they behave as somewhat complex drift bottles.

Methods — During the winters of 1964 and 1965, the Aerial Ice Reconnaissance Branch of the Department of Transport flew numerous missions around Cape Breton Island and adjacent areas. Frequently, the same ice fields were observed on consecutive days and thus were subject to positional studies. Differentiation of the individual ice fields was made possible by a comparison of their typical thickness, concentration, and ridging characteristics. By calculating local wind vectors, the wind-induced drift of the floes can be obtained by theoretical means. Generally, a significant difference occurs between the predicted and observed movements. Ignoring the momentum exchange between individual floes of a field, the positional discrepancy can be attributed to residual (permanent) surface currents. Coastal influence was minimized by choosing ice fields not in direct contact with the shore. The effects of tidal motion were not considered, as the aerial observations and comparisons were made at approximately 24-hr intervals.

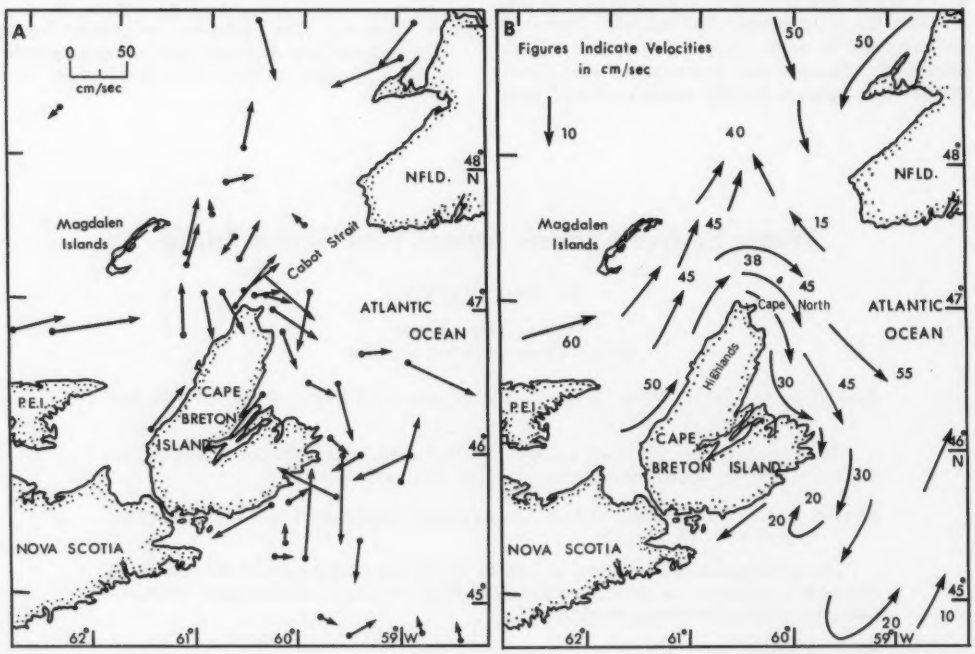

FIG. 1. A, Individual surface current vectors calculated from wind and ice data. B, Composite derived surface circulation around Cape Breton Island.

The method used to estimate the ice field motion resulting from wind drag was essentially the same as that employed by Ingram et al. (1969). Uncorrected geostrophic winds were calculated (Phillips 1963) from surface atmospheric pressure maps at 6-hr intervals with a 1° latitude by 1° longitude grid. The actual wind was assumed to be smaller in magnitude than the geostrophic wind and at an angle of 20° to the left of it (Blackford and Tsang 1964). Meteorological data from eight weather stations (Fox River and Cape Whittle, Quebec; Grindstone Island; Charlottetown, P. E. I.; Sydney, N.S.; and Stephenville, St. Andrews, and Daniels Harbor, Nfld.) were used to compute the appropriate wind reduction factor. The estimated wind stress vector was then obtained from the corrected geostrophic winds. The predicted ice motion was then determined by using Reed and Campbell's (1962) steady-state model, which includes wind stress, water stress, and Coriolis effects. The difference between the observed motion of an ice field and that predicted by the model was attributed to permanent surface currents.

Results — Using the methods described above, a number of surface current velocity vectors were calculated (Fig. 1A). The circulation pattern obtained was generally continuous. However, northwest of Cape North, a complex distribution of currents resulted from the inclusion of three individual drift vectors. Examining the predicted wind conditions, it was found that these three southerly-directed current vectors resulted during southerly wind conditions. The influence of the Cape Breton highlands on the wind drag estimates was thought to be significant near shore and the results questionable. Ignoring the three southerly-directed vectors, a general circulation pattern was obtained as shown in Fig. 1B. The resultant streamlines compare favorably with the spring and limited winter values found by Bumpus and Lauzier (1965). Furthermore, the general flow pattern obtained by El-Sabh and Johannessen (1972) in the region north of Cape North is similar to that obtained in the present study. The only area of marked difference in the streamlines lies adjacent to the Magdalen Islands. The present method, however, gives flow speeds greater by a factor of two than those quoted in El-Sabh and Johannessen. No comparative work is available for most of the data shown in Fig. 1B.

The most interesting features of the derived circulation are the strong outward flow on the southern side of Cabot Strait, with a weak inward center flow; the southwesterly flow off the west coast of Newfoundland; an eddy formation off the southeast coast of Cape Breton Island; and the flow reversal with increasing distance from the southern Cape Breton coastline.

Conclusions — Although this approach to the study of surface currents cannot replace classical methods, it does provide an overall view of the winter circulation around Cape Breton Island. As with all ice drift forecasting models, the assumption of neutral atmospheric stability and simplified roughness parameter-dependence leads to an inexact wind stress field. However, the present technique of estimating the local wind vectors did give favorable results, when compared to the actual values obtained from meteorological stations around the Gulf (Ingram 1967). The significance of melting was minimized by considering fields of chiefly medium or thick winter ice (i.e. thickness greater than 6 inches).

A detailed knowledge of the time-dependent circulation of winter surface currents in the area of Cape Breton Island can only be obtained by a large scale operational program. Until then, indirect methods, as presented here, must be combined with the sparse available data to estimate the overall flow characteristics.

Acknowledgments — This work was supported by the National Research Council of Canada.

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Transatlantic Migration of a Harp Seal, *Pagophilus groenlandicus*

D. E. SERGEANT

Fisheries Research Board of Canada
Arctic Biological Station, Ste. Anne de Bellevue, Que.

SERGEANT, D. E. 1973. Transatlantic migration of a harp seal, *Pagophilus groenlandicus*. J. Fish. Res. Board Can. 30: 124–125.

A harp seal, *Pagophilus groenlandicus*, tagged in the Gulf of St. Lawrence, was recovered at sea off northern Norway at 14 months of age. From tag recoveries, the degree of transfer eastward of young animals from the morphologically most distinct western population of harp seals can be assessed at about 1%, and from the central to the morphologically rather similar eastern population, about 10%.

SERGEANT, D. E. 1973. Transatlantic migration of a harp seal, *Pagophilus groenlandicus*. J. Fish. Res. Board Can. 30: 124–125.

Un phoque du Groenland, *Pagophilus groenlandicus*, étiquetté dans le golfe Saint-Laurent, a été recapturé en mer au large du nord de la Norvège à l'âge de 14 mois. D'après les retours d'étiquettes, on évalue à environ 1% le transfert vers l'est de jeunes phoques provenant de la population de l'ouest, morphologiquement très distincte, et à environ 10% le transfert de phoques de la population centrale vers la population plutôt semblable de l'est.

Received June 12, 1972

UP to now all harp seals (*Pagophilus groenlandicus*) tagged at Newfoundland have been recovered west of Kap Farvel, Greenland (Rasmussen and Oritsland 1964; Sergeant 1965; Popov 1970).

The first tag recovery from an animal which had crossed the North Atlantic Ocean eastward can now be reported.

On May 20, 1970 the Danish fishing vessel *Polarlaks* caught a tagged harp seal on a salmon longline hook at Lat. 70°N, Long. 11°E (80 miles northwest of Andenaes, Norway) (Fig. 1). The seal (Canadian tag no. 10976) proved to have been marked as a pup on March 21, 1969 at 46°30′N 63°W near Prince Edward Island, Canada in the southern Gulf of St.

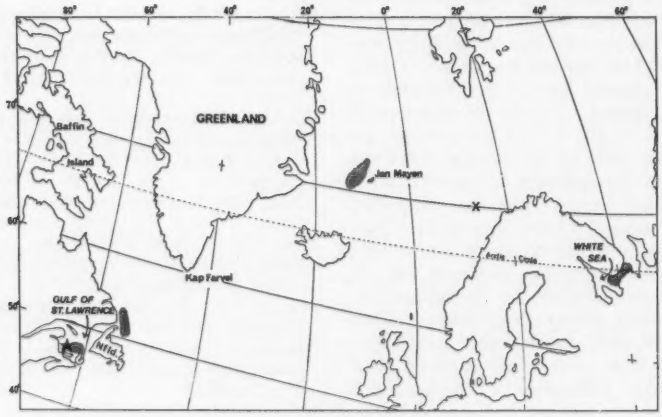

FIG. 1. Whelping grounds of harp seals (*Pagophilus groenlandicus*), and sites of tagging (asterisk) and recovery (cross) of animal described.

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TABLE 1. Known tagging of harp seals (*Pagophilus groenlandicus*) at the three whelping regions in the North Atlantic Ocean.

Agency	Up to year	Newfoundland	Jan Mayen	White Sea
Canada	1970	14,811	–	–
Norway	1963	97	395	31
U.S.S.R.	1968	79	–	2,760
Total		14,987	395	2,791

Lawrence. The tag was a sheep ear-tag of monel metal clamped into the tail.

The recently developed offshore salmon fisheries have now accounted for two harp seals tagged in Canada. The other animal, recovered on Store Hellefiske Bank, Greenland in November 1968, was taken in a salmon driftnet. Most summer recoveries are of harp seals taken by Greenlandic or Canadian Eskimo hunters.

Khuzin (1963) and Yablokov and Sergeant (1963) quantified the morphological differences between harp seals whelping at the three regions of Newfoundland, Jan Mayen, and the White Sea. The latter two populations were closely similar in cranial characters, but Newfoundland animals were more distinct.

Of 86 recaptured harp seals aged 1 or more years, tagged as pups at Newfoundland by Canadian investigators from 1966–69, and recovered during winter or spring sealing, all were from Newfoundland. Rasmussen and Oritsland (1964) give two additional recoveries of the same kind from Norwegian tagging at Newfoundland. According to the data of Rasmussen and Oritsland (1964) there have been eight recoveries in the spring 1 year or later of seals tagged as pups at Jan Mayen. Of these, one crossed to the White Sea. From the data of Sivertsen (1941) and Popov (1970), of six harp seals recovered 1 year or more after being tagged as pups in the White Sea, all were recaptured in the same region.

Recoveries of summering animals tagged as young by Canadian scientists in waters off eastern Canada now total 84, of which 80 came from West Greenland, three from Baffin Island, and the last is the recapture described here. Seals summering in the eastern North Atlantic are in pelagic polar waters in which there is nowadays no sealing. However, from prewar Norwegian tagging in the White Sea reported by Sivertsen (1941), there was one summer recapture, 8 years after tagging. This animal had moved to the northwest side of the Barents Sea, suggesting that transfers from the White Sea to the Jan Mayen stock might occur.

On the basis of these recaptures, the exchange eastward from Newfoundland is of the order of 1%; eastward from Jan Mayen, of the order of 10%, which is in accord with morphological findings. No westward exchange has yet been demonstrated. Drift of young animals from Jan Mayen southwestward might be expected to result in some interchange round Kap Farvel but, as shown in Table 1, the number of animals tagged at Jan Mayen has been rather small in comparison with other areas.

Acknowledgments — I am grateful to Mr S. A. Horsted, Charlottenlund, Denmark for sending first details of the recapture and to Captain Ole Basse Mortensen, Svaneke, Denmark for providing further details.

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A System for Maintaining Midwater Fishes in Captivity

BRUCE H. ROBISON[1]

Hopkins Marine Station
Pacific Grove, Calif. 93950, USA

ROBISON, B. H. 1973. A system for maintaining midwater fishes in captivity. J. Fish. Res. Board Can. 30: 126–128.

A system for maintaining midwater fishes in captivity is described. The system employs a darkened, round-bottom boiling flask containing a structured flow of sea water. Damaging results of the fishes' characteristic escape responses are prevented. Dipnetted specimens of *Tarletonbeania crenularis* were maintained aboard ship and ashore for periods of up to 72 hr.

ROBISON, B. H. 1973. A system for maintaining midwater fishes in captivity. J. Fish. Res. Board Can. 30: 126–128.

L'auteur décrit une méthode permettant de garder en captivité des poissons de moyenne profondeur. Cette méthode est basée sur l'emploi d'un ballon à fond rond avec débit structuré d'eau de mer. On prévient ainsi les blessures qui pourraient résulter de la réaction d'évasion caractéristique du poisson. On a ainsi conservé à bord d'un navire et à terre des *Tarletonbeania crenularis* capturés à l'épuisette, durant des périodes allant jusqu'à 72 heures.

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THE difficulty of maintaining midwater fishes in the laboratory results from the interaction of several factors. Specimens collected by trawling seldom reach the surface alive; those that do survive are usually scaled or skinned or otherwise damaged. Most midwater animals have never contacted a hard substrate and nearly all are equipped with a very strong avoidance–escape response that is keyed to contact, light, and low frequency vibrations. When introduced into standard shipboard aquaria these fishes characteristically destroy themselves by battering against the corners and walls of their containers or they abrade their lower jaws against the bottom in a relentless effort to dive.

The literature contains few references to the survival of midwater fishes in captivity for periods longer than 2–3 hr.

Beebe (1926) and Beebe and Vander Pyl (1944) reported keeping the slender-tailed myctophid *Gonichthys coccoi* alive in shipboard aquaria for up to 36 hr, but this period is by far the longest reported for myctophids and has apparently not been duplicated since 1925. Recent work by Dr Malcolm Gordon (University of California at Los Angeles personal communication) has resulted in the survival of *Myctophum nitidulum* and *Symbolophorus californiensis* for 10–12 hr. An exceptionally hardy bathypelagic species, *Anaplogaster cornuta*, has been kept in laboratory aquaria for many days by Robert Meek and Dr James Childress (University of California at Santa Barbara personal communication). However, this deep water form is relatively rare and atypical of most midwater fishes.

In 1971 a system was developed which has allowed a number of midwater fishes to be maintained at sea and in shore laboratories for extended periods, up to 3 days for one species. The system is portable and may be transported from ship to shore without damage to the specimens. With further development the system should allow prolonged periods of maintenance and provide for direct experimentation upon the specimens.

The system (Fig. 1) consists of a darkened round-bottom boiling flask containing a structured flow of sea water. Flasks of 12- and 50-liters capacity have been used. The flasks provide no corners or flat surfaces to trap the animals. Four 7-mm diameter holes are drilled, 90° apart, around the equator of each flask. A water jet made from glass tubing is placed through each hole and sealed with resin to prevent leakage. One pair of the jets, positioned opposite each other, produces an equatorial current in the manner of the planktonkreisel (Greve 1968). These two jets are directed in the equatorial plane and angled 15° from a plane tangent to the sphere at the point of insertion. The second pair of jets are angled at 60° downward from the equatorial plane and

[1]Present address: Woods Hole Oceanographic Institute, Woods Hole, Mass. 02543, USA.

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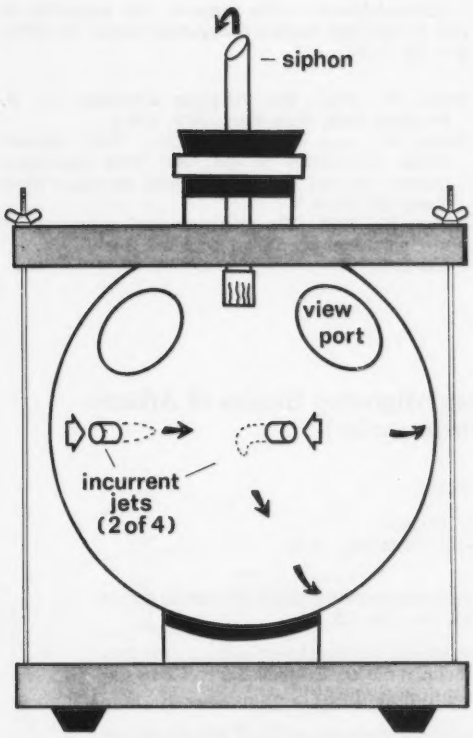

FIG. 1. Diagram of maintenance system. Vessel is a 50-liter round-bottom boiling flask, painted black and soft mounted, with water circulating equatorially and sweeping the bottom.

30° from the tangent. These jets provide currents which sweep the bottom yet are eventually confluent with the equatorial flow.

A vertical drain siphon of 14-mm diameter glass tubing is positioned a few centimeters below the neck of the flask. With a constant inflow, the water depth remains at the level of the siphon opening. This is an open flow system. However, conversion to a closed system is feasible and would allow the incorporation of water temperature control units. When only large organisms are within the flask, a fringed section of rubber tubing is placed at the end of the siphon to prevent capture of the specimens by suction. When small organisms are present, a bag of nylon mesh is tied around the end of the siphon.

A black rubber stopper, bored for the siphon tube, closes the neck of the flask. The system is painted flat black on the outside and down the throat of the neck to stopper depth. Two unpainted areas on the upper half of the flask provide viewing and red-light illumination windows. The windows are sealed with black plastic sheets and tape when not in use. The flask is soft mounted with rubber at the bottom and around the neck between two wooden squares. The upper square has a central hole and there are four threaded brass rods placed at the corners. The lower square is mounted on rubber feet to dampen ship vibrations.

Species tested — The survival times for some of the species that were maintained within the system were:

	Hours until death	Reason
Family Myctophidae		
Tarletonbeania crenularis	72	light leak
Myctophum nitidulum	9	overcrowding
Diaphus theta	12	net damage
Family Melamphaidae		
Poromitra crassiceps	8	net damage
Family Bathylagidae		
Leuroglossus stilbius	24	captured by siphon
Family Anaplogasteridae		
Anaplogaster cornuta	48	net damage

These times represent the most successful runs of at least two fish from each species.

Discussion — When introduced into the system, most fishes require only a few minutes to become relatively quiescent. Water flow across the bottom inhibits the tendency to dive, and the equatorial flow component provides a supporting and directional force. Specimens generally swim in a circular pattern, a few centimeters from the wall and slightly above the equatorial plane. Usually, four to six 7-cm specimens can be introduced without overcrowding; however, this will vary with the sociability of the species.

While feeding by captive fishes has not been observed, on several occasions zooplankters, which were placed in the flask with the fishes, disappeared although there was no apparent source of escape. Spontaneous luminescence has been observed on two occasions when fishes collided with water jets. When red-light illumination is projected through one of the windows and there is no other source of light, the specimens can be observed with no apparent awareness on their part.

The most successfully maintained species, *T. crenularis*, was attracted to the sea surface at night by an underwater light and then dipnetted. This collection technique is the gentlest means of capture and it allows immediate transfer to the system. When the sea water supply system taps the same surface waters from which the specimens came, there is no thermal or pressure shock.

Because many midwater fishes cannot be attracted to the surface, maintenance of the deeper-dwelling forms depends on the occasional capture of specimens in good condition. The number of uninjured specimens collected could be increased by the wider use of knotless mesh for midwater trawls and the development of larger codends which cushion their catches. An alternative collection technique which has yielded several deepwater fishes in relatively good condition is the use of 1-m zooplankton nets with .333-mm nylon bag codends in short-duration vertical hauls.

Acknowledgments — The research was supported in part by National Science Foundation Grants GB 27254 and GB 31559.

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A Modified Stake Net for Collecting Migrating Smolts of Atlantic Salmon (*Salmo salar*).

G. M. HARE[1]

Department of Biology
University of New Brunswick, Fredericton, N.B.

HARE, G. M. 1973. A modified stake net for collecting migrating smolts of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 30: 128–129.

A modified stake net for collecting smolts of Atlantic salmon (*Salmo salar*) is described. The net is efficient for trapping smolts and, in addition, it practically eliminates problems with mortality of captured fish and accumulation of debris in the trap.

HARE, G. M. 1973. A modified stake net for collecting migrating smolts of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 30: 128–129.

Une étente a été modifiée pour la collection de smolts de saumon atlantique (*Salmo salar*) en voie de migration. Le filet est efficace pour la capture des smolts. De plus, on élimine dans une large mesure les mortalités chez les poissons capturés, ainsi que les problèmes de collection de débris dans l'étente.

Received July 25, 1972

MIGRATING smolts of Atlantic salmon (*Salmo salar*) were collected from six large tributaries of the Miramichi River system, New Brunswick, as part of a parasitological investigation in 1970 and 1971. Portable fyke nets of conventional design (Lagler 1952) were used in 1970. Catches with these nets depend on guiding downstream migrants into a trap which has its entrance at the downstream apex of two converging wing nets. However, several observations have shown that most smolts, when guided to the trap, avoid entry by swimming upstream. Based on these observations, a stake net (Went 1964) was modified and used with success for collecting smolts in 1971.

Description of net — The net (Fig. 1) consists of a rectangular trap (7 × 9 × 4 ft) and two detachable wings (50 × 4 and 14 × 4 ft). The trap and wings are attached to four 6-ft cast-iron pickets 1 inch in diameter. The bottom, sides, and throat of the trap are constructed of nylon netting, stretched mesh 0.25 inch square, and the wings of similar netting, mesh 0.50 inch square. Both trap and wings are mounted on 0.25 inch nylon rope. The throat, situated at the downstream end of the trap, is 3 ft deep and tapers to an entrance 10 inches wide. An adjustable throat string and two adjustable throat lines maintain the dimensions of the entrance.

[1]Present address: Environment Canada, Fisheries Service, P. O. Box 5667, St. John's, Nfld.

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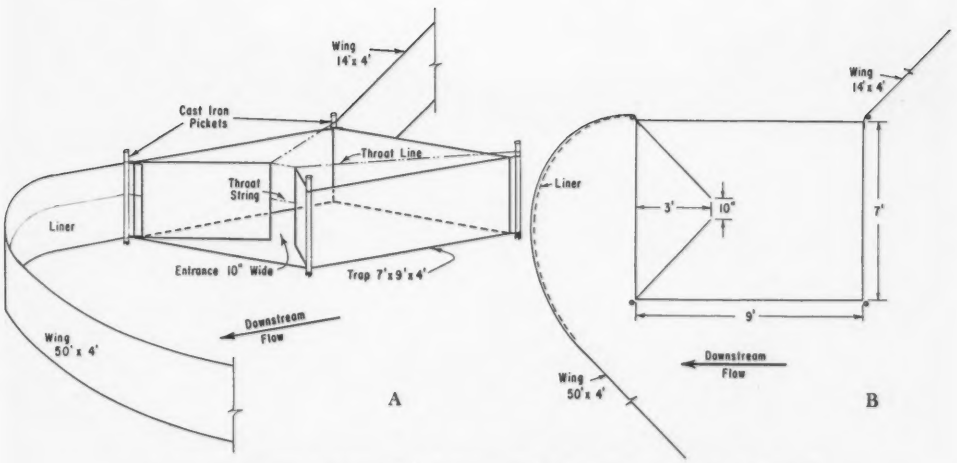

FIG. 1. (A) Diagram of modified stake net used to collect smolts, (B) plan view.

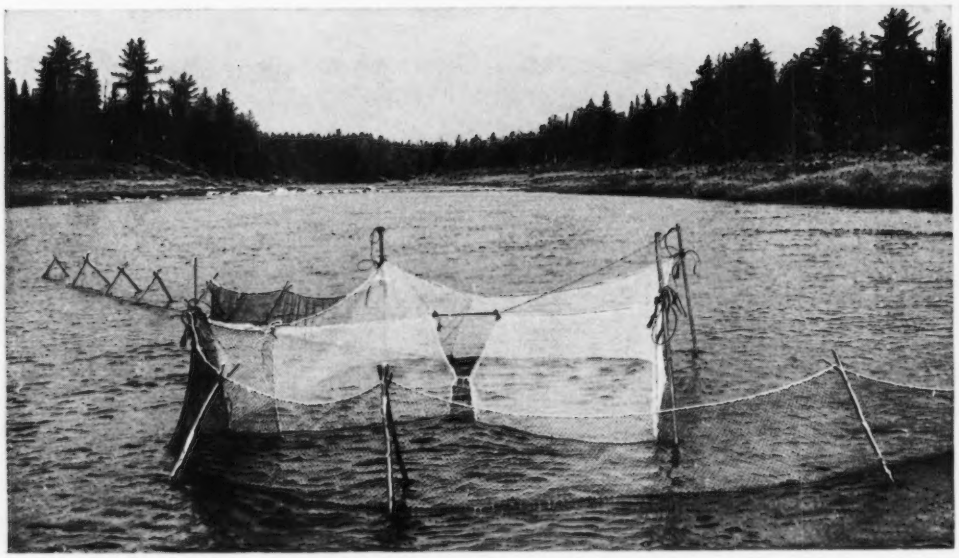

FIG. 2. Stake net in fishing position.

Installation and operation — To set the net (Fig. 2), the upstream pickets are driven into the stream bottom and the upper end of the trap is attached; this is followed by driving the downstream pickets and attaching the lower end of the trap. When the trap is in position, the wings are attached to pickets on either side of the trap, depending upon the fishing position desired. The wings, weighted with rocks, are held upright by forked hardwood stakes and placed at approximately 45° to the current. To obtain optimum catches, additional lengths of wings may be added when water depths permit, and a wing liner (mesh 0.25 inch square) may be added to the wing at the downstream end of the trap.

To remove fish from the net, the bottom of the downstream end of the trap is elevated and tied on pickets to prevent captured fish from escaping. Similarly, the upper end of the trap is elevated and tied. Fish are then removed with a dip net, and the net may be reset by lowering the trap.

Efficiency — The net worked well in depths up to 3.5 ft and current flow to 600 ft^3/sec. Seven sets with the present net yielded an average of 315 smolts (range 34–1256) from the Miramichi River tributaries. Other important factors included negligible mortality of captured fish and the elimination of debris from the trap.

The trapping efficiency of the net presumably results from the positions of the wings, which guide fish into a confined area at the downstream end of the trap. Smolts respond to this apparent confinement by swimming directly upstream and, hence, into the trap. Also, the partial diversion of stream flow away from the trap entrance may account for the low mortality of captured fish and the elimination of debris from the trap.

Acknowledgments — I wish to express my thanks and appreciation to the following: the Fisheries Research Board for scholarships and help from personnel at the Curventon and Trout Brook counting-fences; the Atlantic Salmon Association for financial support during the summers of 1970 and 1971; the National Research Council for assistance through Operating Grant A258 to Dr M. D. B. Burt; Messrs G. W. Cooper and W. R. Currie, Resource Development Branch, Fisheries Service, Department of the Environment, Newcastle, N.B., for advice on netting procedures; Mr P. W. G. McMullon, Fisheries Research Board, St. Andrews, N.B., for illustrations; and Drs M. D. B. Burt and A. J. Wiggs, Department of Biology, University of New Brunswick, for reading the manuscript.

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Evidence for Homing in the Mosshead Sculpin (*Clinocottus globiceps*)[1]

JOHN M. GREEN

Marine Sciences Research Laboratory
Memorial University, St. John's, Nfld.

GREEN, J. M. 1973. Evidence for homing in the mosshead sculpin (*Clinocottus globiceps*). J. Fish. Res. Board. Can. 30: 129–130.

Observations on 86 tagged mosshead sculpins (*Clinocottus globiceps*) show that this species exhibits fidelity to specific tide pools and returns to them when displaced to other tide pools.

GREEN, J. M. 1973. Evidence for homing in the mosshead sculpin (*Clinocottus globiceps*). J. Fish. Res. Board. Can. 30: 129–130.

Le marquage de 86 *Clinocottus globiceps* démontre que cette espèce est fidèle à un étang à marée spécifique et y retourne lorsque transplantée dans d'autres étangs.

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[1]Contribution No. 355 from Department of Biology; Contribution No. 116 from Marine Sciences Research Laboratory.

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HOMING behavior has been reported for a number of northeastern Pacific intertidal fishes (Williams 1957 and Green 1971a). This note reports on tagging experiments that were conducted to determine whether the mosshead sculpin (*Clinocottus globiceps*) would remain in specific tide pools and return to them when displaced to other pools. The study was conducted on the southwest coast of Vancouver Island, B.C., near the mouth of San Juan Inlet. This site was occupied by the author on a nearly continuous basis from June 1965 to August 1967. During this period, tagging studies of the mosshead sculpin were conducted as part of an investigation of the distribution and behavior of tidepool cottids (Green 1967). The study site and the intertidal distribution of *C. globiceps* have been described in an earlier paper (Green 1971b). In June and July of 1971 the author returned to the study site and did futher tagging of the mosshead sculpin.

Materials and methods — The same methods as described by Green (1971a) were used. Fish were caught at low tide during daylight in baited minnow traps or with small dipnets at night, when this species occupies exposed positions in pools. Tags were embroidery beads of various colors attached to the middorsal musculature with monofilament nylon line. No anesthetic was used during tagging and fish were measured and sexed at the time of tagging.

Tagged fish were released both into the tide pool from which they were captured and into other tide pools. A total of 39 mosshead sculpins (11 male and 28 female) were tagged and released into the four pools from which they were collected. Another 47 mosshead sculpins (28 male and 19 female) were tagged and released into other pools. During the study there were nine separate transplant experiments involving six different tide pools. Since one cannot be sure that a tagged fish has left a pool between consecutive recaptures, only the latter releases conclusively demonstrate that a species exhibits homing behavior.

Usually, tagged fish were released on the low tide of capture but in a few experiments surf conditions necessitated that they be held until the next low tide. The average transplant release distance was 10 m and all tide pools used in this study were located between the 1- and 3-m tide levels.

Recoveries were made with minnow traps and by visual observations of pools during the day and night. The trapping and observation of tide pools commenced on the day following a release and continued for at least a month. The effectiveness of these recovery methods (Green 1971a, b) makes it likely that nearly all tagged fish in the study area were recaptured or observed.

Results and conclusions — Of the 36 fish recovered after being released in tide pools where they were captured, 34 (94%) were taken in these tide pools. These data include only recoveries made at least 2 weeks following release, and only the initial recovery of a specimen after this period is included although many of the fish were caught on numerous occasions.

Of the 32 fish recovered after being displaced, 30 (94%) were taken in the tide pools where they were captured. Again, most of the recovered fish were caught numerous times, but only the initial recovery of a transplanted fish is included in the above data.

Using the combined data for all the transplant releases, there was no significant difference ($P = 0.05$) in homing success between males and females. This was also true for the home pool releases. With respect to fish length, there was no significant difference in homing success between 5-mm size groups over the size range (50–90 mm) tagged.

The length of time that individuals spend in one tide pool cannot be calculated from decreases in tag returns because tag loss is a significant factor. Tagged fish may also be more susceptible to predation. However, 22 (59%) of the tagged fish which were released into their home pools were still in those pools 3 months later, and five tagged fish were observed in the same tide pool for 6 months.

The present report adds another species to the number of marine fish which have been shown to exhibit homing behavior. However, not enough is known about the high tide distribution of *C. globiceps* to speculate about what orientation mechanism is involved.

Acknowledgments — Drs V. J. Steele and R. Strickler offered helpful comments on the manuscript and Mr V. Pepper did the statistical analyses.

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An Early Record of the Sea Lamprey (*Petromyzon marinus*) From Lake Ontario

JOHN G. I. LARK

Department of Zoology
University of Toronto, Toronto, Ont.

LARK, J. G. I. 1973. An early record of the sea lamprey (*Petromyzon marinus*) from Lake Ontario. J. Fish. Res. Board Can. 30: 131–133.

An 1835 record of the sea lamprey (*Petromyzon marinus*) in Lake Ontario is related to a hypothesis that it entered the lake through the Erie Canal after 1819.

LARK, J. G. I. 1973. An early record of the sea lamprey (*Petromyzon marinus*) from Lake Ontario. J. Fish. Res. Board Can. 30: 131–133.

Un signalement, daté de 1835, de la grande lamproie marine (*Petromyzon marinus*) dans le lac Ontario est lié à l'hypothèse que la grande lamproie marine a pénétré dans le lac par le canal Erié après 1819.

Received March 15, 1972

AN excerpt from the Clendenan Volume of the diaries of Charles Fothergill (undated p. 50–51) contains the earliest known record (Fig. 1) of the sea lamprey (*Petromyzon marinus*) in Lake Ontario. The record suggests a breeding population of sea lamprey in Duffin's Creek (43°49′W, 79°02′N), just east of Toronto, in May 1835.

Aron and Smith (1971) have proposed that the sea lamprey invaded Lake Ontario by way of the eastern half of the Erie Canal, opened in 1819. Smith (1972) noted its occurrence in the Finger Lakes, which are continuous with the Erie Canal System, in the 1870s. If Fothergill's statements on extensive damage to earthen dams are reliable, then I suggest that the sea lamprey was in Lake Ontario prior to the opening of the Erie Canal, or that it invaded the lake more rapidly than proposed by Smith (1972). W. B. Scott (personal communication) postulates that the sea lamprey invaded these waters following Pleistocene glacial action and have been there ever since, but no direct evidence in support of this has been found.

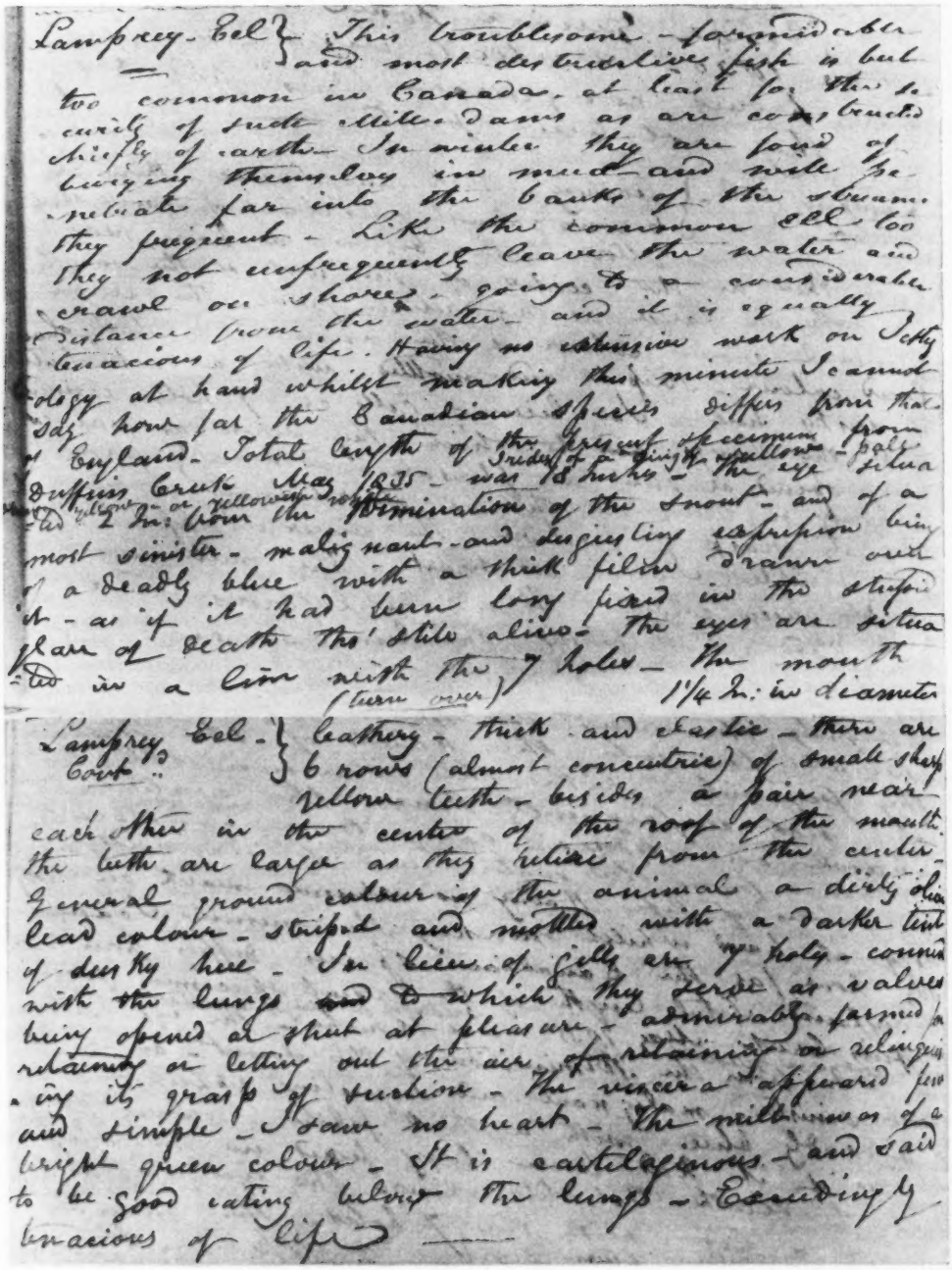
Fothergill's contributions to natural history have been reviewed by Baillie (1944). Born in England, he was highly respected (e.g. for his notes on the birds of Upper Canada). The meticulous nature of his descriptions and his obvious scruples with respect to describing first hand experience have been noted. As a conservationist, Fothergill succeeded in having passed an Act for the better preservation of salmon in Ontario in 1830, only to have the Executive of Upper Canada veto it (Baillie 1944).

Printed in Canada (J2475)

Fothergill's description of *P. marinus* is in general consistent with present knowledge of its morphology (see Table 1) and ecology. The reference to the destructive nature of sea lamprey appears somewhat fanciful but may well relate to the ability of adult lamprey, in their upstream spawning migration, to wriggle through mud and cracks or crevices of small beaver dams or man-made dams. Scott and Crossman (1973) state further that sea lamprey are capable of surmounting nearly vertical barriers of 1½–2 m high, where the current is not too strong. This could relate to the statement that lamprey "leave the water and come on shore." Fothergill may have known about the burrowing habits of the ammocoetes (Vladykov 1950) but it is unlikely that ammocoetes could pose any threat to mill dams, even in early years when mill dams were poorly constructed.

Fothergill's description of external coloration agrees closely with that of Scott and Crossman (1973). The overall size indicates that the specimen must have been *P. marinus* (see Table 1). The relation of buccal disc diameter to total length is inconsistent with the literature. The values for this relation vary from 6.1 to 9.3% (Scott and Crossman 1973; Slastenenko 1958; Vladykov 1949). W. B. Scott (personal communication) explains the discrepancy as follows: in an unpreserved specimen, after rigor mortis has set in, the body becomes flaccid and tends to shrink slightly; the tough horny teeth of the buccal disc, however, act as a "beaded skirt" to retain the original diameter of the buccal disc.

Fothergill's description of the milt as being green suggests that he had mistaken the liver as being the milt. This inference is borne out by the fact that



Lamprey - Eel } This troublesome - formidable
and most destructive fish is but
two common in Canada - at least for the fa-
cility of such Mill Dams as are constructed
chiefly of earth - In winter they are found of
burying themselves in mud and will pe-
netrate far into the banks of the stream
they frequent - Like the common Eel too
they not unfrequently leave the water and
crawl on shore - going to a considerable
distance from the water - and it is equally
tenacious of life. Having no ichnion work on Ichy
ology at hand whilst making this minute I cannot
say how far the Canadian Species differs from that
of England - Total length of the present specimen - from
Duffins Creek May 1835 - was 18 inches - the eye situa
ted 2 In: from the termination of the snout - and of a
most sinister - malignant - and designing expression being
of a deadly blue with a thick filmy drawn over
it - as if it had been long fixed in the stupi
glare of death tho' still alive - The eyes are situa
ted in a line with the 7 holes - The mouth
(Turn over) 1¼ In: in diameter

Lamprey Eel - } leathery - thick and elastic - there are
Cont: } 6 rows (almost concentric) of small sharp
yellow teeth - besides a pair near
each other in the center of the roof of the mouth
the teeth are large as they retire from the center -
General ground colour of the animal a dirty olive
lead colour - striped and mottled with a darker tint
of dusky here - In lieu of gills are 7 holes - comm
with the lungs and to which they serve as valves
being opened & shut at pleasure - admirably formed for
retaining or letting out the air - retaining or relinquis
ing its grasp of suction - The viscera appeared few
and simple - I saw no heart - The milt was of a
bright green colour - It is cartilaginous - and said
to be good eating being the lungs - Exceedingly
tenacious of life -

FIG. 1. Excerpt from the Clendenan volume of the diaries of Charles Forthergill (p. 50–51).

TABLE 1. Some meristic characters of the four lamprey species recorded in the Great Lakes (after Vladykov 1949) and Fothergill's specimen.

Species	Total length (*mm*)	buccal disc diameter × 100 / total length
Ichthyomyzon fossor	109–150	3.6–4.0
Ichthyomyzon unicuspis	89–312	9.1–11.4
Entosphenus lamotenii	112–187	3.6–4.8
Petromyzon marinus	135–528[a]	8.5–9.3
Fothergill's	457	14.4

[a]This is maximum for landlocked specimens; anadromous specimens are found up to 860 mm.

the sea lamprey does not feed during the spawning migration and the intestinal tract reduces in size until it is a mere hollow thread. The color of the liver changes from reddish-orange to green. W. B. Scott (personal communication) states that the texture of the liver in an unpreserved specimen would resemble that of milt.

The only other references Fothergill makes to "lampreys" is found in the McGillivray Volume (p. 30) of his diaries. There he lists fishes of Lake Ontario as follows: "Eels, Lampreys, Perch, Black Bass, Silver Bass, Sunfish, Mullet, Red Mullet, Suckers, Catfish, Garfish, Chub, Minnow, Crawfish, Shad (very rare), Sturgeon, Salmon, Salmon trout, Whitefish, Herring, Pickerel." This list was very likely formulated in 1817 judging from the dates of the preceding and following entries. In it he includes "Lampreys;" no further description is given and it is possible that he is referring to one or a combination of the other three species found in Lake Ontario. "Shad (very rare)" could refer to the alewife (*Alosa pseudoharengus*) or the gizzard shad (*Dorosoma cepedianum*). The "Herring" is presumably *Coregonus artedii*. He did not include any of the smaller coregonines unless perhaps he identified them as "shad."

Acknowledgments — Thanks to H. A. Regier and W. B. Scott for information and assistance with this note, and to S. H. Smith for a helpful review.

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1950. Larvae of eastern American lampreys (Petromyzontidae). Dep. Fish. Prov. Que. Contrib. No. 29: 95 p.

The following is a transcription: Lamprey-eel} This troublesome-formidable and most destructive fish is but too common in Canada, at least for the security of such Mill dams as are constructed chiefly of earth. In winter they are fond of burying themselves in mud and will penetrate far into the banks of the streams they frequent. Like the common eel too they not unfrequently leave the water and crawl on shore — going to a considerable distance from the water — and it is equally tenacious of life. Having no extensive work on Ichtyology at hand whilst making this minute I cannot say how far the Canadian species differs from that of England. Total length of the present specimen from Duffins Creek May 1835 — was 18 inches — [Sides or hides] of a dingy yellow — pale orange-yellow or yellowish white. The eye situated 2 in. from the termination of the snout — and of a most sinister — malighant — and disgusting expression being of a deadly blue with a thick film drawn over it — as if it had been long fixed in the stupid glare of death tho' still alive. The eyes are situated in a line with the 7 holes — The mouth 1¼ in. in diameter, leathery thick and elastic — there are 6 rows (almost concentric) of small sharp yellow teeth — besides a pair near each other in the center of the roof of the mouth. The teeth are larger as they retire from the center. General ground colour of the animal a dirty olive lead colour — striped and mottled with a darker tint of dusky hue. In lieu of gills are 7 holes connected with the lungs to which they serve as valves being opened or shut at pleasure — admirably formed for retaining or letting out the air of [for] retaining or relinquishing its grasp of suction. The viscera appeared fine and simple. I saw no heart. The milt was a bright green colour. It is cartilaginous and said to be good eating below the lungs. Exceedingly tenacious of life.

Mesopelagic Fishes from the Gulf of Maine and the Adjacent Continental Slope[1]

JOHN A. MUSICK

Virginia Institute of Marine Science
Gloucester Point, Va. 23062, USA

MUSICK, J. A. 1973. Mesopelagic fishes from the Gulf of Maine and the adjacent continental slope. J. Fish. Res. Board Can. 30: 134–137.

Otter-trawl records and midwater-trawl catches suggested that some mesopelagic fishes are permanent members of the midwater fauna of the Gulf of Maine rather than strays. *Benthosema glaciale*, *Ceratoscopelus maderensis*, *Myctophum punctatum*, and *Maurolicus muelleri*, cold water or ubiquitous species, occur in the Gulf throughout the year, and warmwater species derived from adjacent Slope Water offshore were captured in the Gulf during the summer and fall. Nine species of mesopelagic fishes are here recorded from the Gulf for the first time.

MUSICK, J. A. 1973. Mesopelagic fishes from the Gulf of Maine and the adjacent continental slope. J. Fish. Res. Board Can. 30: 134–137.

Benthosema, *glaciale*, *Ceratoscopelus maderensis*, *Myctophum punctatum* et *Maurolicus muelleri*, espèces d'eau froide ou ubiquistes, se trouvent dans le golfe du Maine à l'année longue, alors que des espèces d'eau chaude provenant des eaux de pente du large sont capturées dans le golfe en été et en automne. Neuf espèces de poissons mésopélagiques sont signalées pour la première fois dans le golfe du Maine.

Received April 17, 1972

THE purpose of this study was to determine whether mesopelagic fishes occur in the Gulf of Maine as rare strays or as normal components of the fauna. Previously, only 12 species of mesopelagic fishes were known from the Gulf of Maine (Bigelow and Schroeder 1953; Leim and Day 1959) and they were considered to be strays from beyond the continental shelf. The finding of myctophids (*Ceratoscopelus maderensis*) in the stomachs of haddock (*Melanogrammus aeglefinus*) from the northern edge of Georges Bank (R. Livingstone, National Marine Fisheries Service, Woods Hole Laboratory, personal communication) and personal observations of otter-trawl catches suggested that mesopelagic fishes were not rare in the Gulf.

In this study, otter-trawl data collected from three vessels in the Gulf of Maine by the U.S. National Marine Fisheries Service (N.M.F.S.) at the Woods Hole Laboratory between 1955 and 1966 were examined for records of mesopelagic fishes. Also, collections of mesopelagic fishes stored at the Woods Hole Laboratory were examined and during two cruises on R.V. *Albatross IV* in 1965 several Isaacs-Kidd midwater trawl (I.K.M.T.) collections were made in the Gulf and along the adjacent continental slope. Additional I.K.M.T. data from the Gulf was supplied by Richard Backus at the Woods Hole Oceanographic Institution.

The trawl used in the R. V. *Albatross IV* survey, for groundfish, was a 10-ft (3.05-m) I.K.M.T. (Isaacs and Kidd 1953). The trawl was fully lined (12.7-mm stretch mesh) and an M.S. Bathykymograph model T-1C was attached to the depressor of the net to record depth and time at Stations 80A and 89A (Fig. 1). Station data for R.V. *Albatross IV* (I.K.M.T.) and otter trawl collections and for I.K.M.T. collections made by R. H. Backus (R.V. *Chain* cruise) are given in Table 1. In addition, N.M.F.S. data collected at 34 otter trawl and 2-m net stations listed mesopelagic fishes only to family or suborder from R. V. *Albatross III* cruises 65, 75, 76, 82, 118, and 119, on R. V. *Delaware* cruises 59–13, 60–12 and 61–19, and R. V. *Albatross IV* cruises 63–5, 63–7, 64–1, 64–10, 64–13, 65–2, 65–10, 65–14, and 66–1. These data are summarized for analysis in Fig. 1.

Most specimens from R. V. *Albatross IV* stations 80A, 89A, and x have been deposited in the collections of the Virginia Institute of Marine Science.

Species captured — Following the species name are the R. V. *Albatross IV* and R. V. *Chain* station(s) at which the species was taken and, in parentheses, the number of specimens and range of standard lengths.

[1]Virginia Institute of Marine Science contribution No. 506.

Printed in Canada (J2503)

In the Anguilliformes: the derichthyid *Derichthys serpentinus* Gill, x (1, 204 mm); the serrivomerid *Serrivomer beani* Gill and Ryder, 80-A (2, about 220 mm), 89-A (2, about 200 mm); the nemichthyid, *Nemichthys scolapaceus* Richardson, x (4, 800–900 mm), 80-A (4, 490–760 mm), 89-A (4, 235–530 mm); the nessorhamphid, *Nessorhamphus ingolfianus* Schmidt, 80-A (1, 262 mm), 89-A (1, 156 mm).

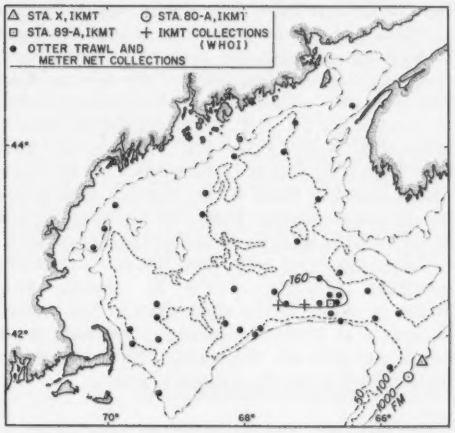

FIG. 1. Positions where mesopelagic fishes were captured in the Gulf.

In the Salmoniformes: the bathylagid, *Bathylagus bericoides* (Borodin), x (1, 77 mm); the sternoptychid, *Polyipnus asteroides* Schultz, x (1, 28 mm); the gonostomatids, *Gonostoma elongatus* Gunther, x (1, 123 mm), 80-A (2, 149, and 160 mm), 89-A (1, 118 mm); *Maurolicus muelleri* (Gmelin), x (2, 19, and 25 mm), 80-A (2, 30, and 31 mm), 43 (2, 31, and 33 mm); and *Valenciennellus tripunctulatus* (Esmark), 80-A (1, 18 mm); the chauliodontid, *Chauliodus sloani* Bloch and Schneider, 80-A (4, 73–113 mm), 89-A, (1, 89 mm); the stomiatid, *Stomias boa ferox* Reinhardt, x (5, 84–203 mm), 80-A (8, 95–116 mm), 89-A (10, 70–116 mm).

In the Myctophiformes, the myctophids, *Benthosema glaciale* (Reinhardt), x (102, 17–63 mm), 80-A (219, 15–59 mm), 89-A (29, 21–48 mm), 69 (1, 35 mm), 58-A (19, 43–69 mm), 60 (2, 74 and 80 mm), RHB-850 (5, 21–54 mm), RHB-854 (9, 40–45 mm); *Ceratoscopelus maderensis* (Lowe), x (18, 52–65 mm), 80-A (24, 18–65 mm), 89-A (1, 60 mm), 69 (1, 63 mm), 59 (3, 56–59 mm), 60 (16,

TABLE 1. Station data for mesopelagic fishes collected in the Gulf.

Station	Cruise	Date	Time	Longitude	Latitude	Comments
			With I.K.M.T. from R. V. *Albatross IV*			
x	65–10	July 19, 1965	2201-2301 (EDT)	41°42′N	65°25′W	1 tow, duration 1 hr, sounding 1110 fath, fishing depth (est.) 100 fath.
80A	65–14	Oct. 16, 1965	2045-2240 (EDT)	41°35′N	65°40′W	1 tow, 1 hr, sounding 1000 fath, fishing depth (T.D.R.) 100 fath.
89A	65–14	Oct. 18, 1965	0110-0535 (EDT)	42°20′N	66°45′W	5 tows, 20 min, sounding 164–200 fath fishing depth (T.D.R.) 11 fath, 28 fath, 39 fath, 60 fath, 88 fath.
			By otter trawl from R. V. *Albatross IV*			
69	64–1	Jan. 25, 1964		42°17′N	65°45′W	1 tow, 1 hr, depth 105 fath.
58A	65–2	Nov. 13, 1965		42°29′N	66°12′W	1 tow, 1 hr, depth 124 fath.
43	66–1	Jan. 25, 1966		44°15′N	67°17′W	1 tow, 1 hr, depth 100 fath.
59	66–1	Feb. 2, 1966		42°24′N	66°42′W	1 tow, 1 hr, depth 175 fath.
60	66–1	Feb. 3, 1966		42°10′N	66°35′W	1 tow, 1 hr, depth 75 fath.
			With I.K.M.T. by R. Backus from R. V. *Chain*, Cruise 32			
RHB-850		Sept. 12, 1962	0900-1235 (EDT)	42°18′N	67°28′W	Sounding 170 fath, fishing depth: struck bottom.
RHB-854		Sept. 13, 1962	0555-1025 (EDT)	42°19′N	67°06′W	Sounding 150 fath, fishing depth (est.) 88 fath.

60–70 mm); *Diaphus dumerili* Bleeker, x (1, 21.5 mm); *Hygophum hygomi* (Lutken), x (4, 30–39 mm); *Lampanyctus alatus* Goode and Bean, 80-A (1, 34 mm); *Lampanyctus crocodilus* (Risso), 80-A (1, 45 mm); 89-A (1, 48 mm); *Lobianchia dofleini* (Zugmayer), x (119, 19.5–39.0 mm), 80-A (30, 23.5–36.0 mm), 89-A (2, 33, and 34 mm); *Myctophum punctatum* Rafinesque, x (1, 27 mm), 89-A (1, 47 mm), 59 (6, 66–83 mm); *Notoscopelus kroyeri* (Malm), x (2, 25, and 34 mm), 80-A (2, 48, and 53 mm); *Notoscopelus resplendens* (Richardson), 80-A (1, 57 mm); and *Symbolophorus veranyi* (Moreau), x (1, 50 mm); the paralepidid *Notolepis rissoi kroyeri* Lutken, 80-A (22, 50–162 mm), 89-A (7, 56–116 mm).

Nine of the species are here reported for the first time from the Gulf of Maine: *Gonostoma elongatum, Benthosema glaciale, Ceratoscopelus maderensis, Lobianchia dofleini, Lampanyctus crocodilus, Myctophum punctatum, Notolepis rissoi kroyeri, Serrivomer beani,* and *Nessorhamphus ingolfianus.*

Occurrence in the Gulf of Maine — Mesopelagic fishes occur more commonly in the Gulf of Maine than heretofore realized. Thirty-four otter trawl and 2-m net collections of mesopelagic fishes identified only to family or suborder (Fig. 1) included 28 stations with myctophids, seven with gonostomatids, two with paralepidids, and two with stomiatoids.

The inshore extension of the mesopelagic fauna (which migrates vertically daily) is limited by depth. Almost 90% of otter-trawl captures of mesopelagic species were at depths greater than 75 fath (Fig. 1). Halliday (1970) reported the shallowest pelagic day-time capture of *Benthosema glaciale* in deep water over the Nova Scotian slope to be 90 fath. This species is the most abundant mesopelagic species in the Gulf of Maine and probably accounts for many of the records in Fig. 1. Most captures of mesopelagic species in the Gulf at depths shallower than 75 fath have been along precipitous slopes. The topography of the Gulf of Maine (Bigelow 1928) is such that a Y-shaped trough deeper than 100 fath provides access by mesopelagic fishes from the eastern channel into the Gulf to within 20 miles of Cape Cod and 25 miles of the northern coast of Maine (Fig. 1).

Discussion — Only the boreal or ubiquitous species, *B. glaciale, C. maderensis, M. punctatum,* and *M. muelleri,* were captured in the Gulf of Maine during the winter, and probably tropical and warm-water species are excluded from the Gulf at that time unless they abandon their daily vertical migrations and remain near the bottom. In winter, surface temperatures drop as low as 1 C in the Gulf. Bottom temperatures remain at 5–7 C (Bigelow 1928) because of the moderating influence of intruding Slope Water (McLellan 1957). The predominance of *B. glaciale* in I.K.M.T. catches from the Gulf and from the autumn station (80-A) on the continental slope agrees with the findings of Halliday and Scott (1969). They found *B. glaciale* to be predominant in the mesopelagic fauna in the Coastal Current at the edge of the Scotian Shelf. Similarly, the predominance of *Lobianchi dofleini* in the summer I.K.M.T. catch (Station x) from the continental slope agrees with Backus et al. (1970) who found *L. dofleini* predominant in the mesopelagic fauna in Slope Water over the abyssal plain seaward of the Scotian Shelf. The boundary between Slope Water and the Coastal Current is marked by a hydrographic front with steep thermal and salinity gradients (McLellan 1957). In summer, Slope Water is closer to the shelf than in late autumn (Colton et al. 1968). Consequently the catch at Station x was probably from Slope Water and that at Station 80-A from the Coastal Current.

Conclusions — Examination of otter trawl records and I.K.M.T. catches suggests that mesopelagic fishes occur in the Gulf of Maine not merely as strays but as permanent members of the midwater fauna. The mesopelagic fauna in the Gulf of Maine is basically the same as that found by Halliday and Scott (1969) on the deep Scotian Shelf in Coastal Current water. This fauna shares many elements with that of the Slope Water but appears to differ in predominance of species. Additional midwater trawling with closing nets is necessary to better define the distribution and frequency of occurrence of mesopelagic fishes in the Gulf of Maine and to examine the apparent differences between the mesopelagic faunas of the Coastal Current and Slope Water.

Acknowledgments — Thanks are due to R. Edwards, former assistant director of the N.M.F.S. Laboratory at Woods Hole, Massachusetts, who made arrangements for me to participate in cruises aboard R. V. *Albatross IV,* and provided research space at the laboratory and unpublished N.M.F.S. data. Further acknowledgment goes to R. Fritz for his help in the field; R. Backus for access to his unpublished data; B. Nafpaktitis for identification of *Lobianchia* and *Diaphus* and especially to J. Craddock for identification of the remainder of the material. This research was partially supported by a grant to the Harvard Committee on Evolutionary Biology (Principal Investigator, R. C. Rollins), National Science Foundation G1927 and GB3167.

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A Genetic Analysis of Hatching Time in Steelhead Trout (*Salmo gairdneri*)

J. D. MCINTYRE AND J.-M. BLANC

Oregon Cooperative Fishery Unit
Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oreg. 97331, USA

MCINTYRE, J. D., AND J.-M. BLANC. 1973. A genetic analysis of hatching time in steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 137–139.

The elapsed time from fertilization through hatching for steelhead trout (*Salmo gairdneri*) was obtained from groups of eggs that were fertilized according to a factorial breeding plan in which 24 males were each mated to each of 10 females. Data were obtained from the eggs of 240 crosses held in an incubator through hatching and from replicated crosses held in an incubator for most of the incubation period, then in troughs. Estimated heritabilities for hatching time were 0 and 0.23, respectively, for these data, the differences being of additive genetic origin. Because of a significant genotype–environment interaction, it was suggested that hatching may have been controlled by different genetic mechanisms in the two environments.

MCINTYRE, J. D., AND J.-M. BLANC. 1973. A genetic analysis of hatching time in steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 137–139.

Nous avons mesuré le temps écoulé entre la fécondation et l'éclosion dans des groupes d'oeufs de truite steelhead (*Salmo gairdneri*), qui avaient été fécondés selon un schéma de croisement factoriel, en accouplant 24 mâles et 10 femelles de toutes les façons possibles. Nous avons obtenu deux séries de mesures: (1) l'une relative aux oeufs qui, issus des 240 croisements, ont été maintenus dans un incubateur jusqu'à éclosion, et (2) l'autre relative aux oeufs qui, issus des mêmes croisements, ont été placés dans un incubateur pendant la majeure partie de la période d'incubation et ensuite dans des auges. A partir de ces mesures, nous avons estimé à 0 et 0.23, respectivement, l'héritabilité de la durée d'incubation, les différences étant d'origine génétique additive. La présence d'une interaction génotype–milieu significative nous a amenés à suggérer que l'éclosion n'était peut-être pas réglée par les mêmes mécanismes génétiques dans les deux environnements.

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THE amount of time after fertilization that fish eggs hatch can be altered by such variables as temperature, dissolved oxygen, light, and salinity (see reviews by Blaxter 1969 and Smith 1957). Although it is known that biological variability results from genetic as well as environmental influences, the relative importance of these factors is generally not considered. The objective of this study was to determine the magnitude of any additive genetic component of variance in hatching time for steelhead trout (*Salmo gairdneri*).

Methods — During the course of development of steelhead trout eggs that were being incubated to provide fish for other studies, the number of eggs that hatched each day during the hatching period was recorded. These records were obtained from the progeny produced by combining eggs from 10 females with sperm from 24 males in the 240 possible combinations.

The matings were accomplished on May 28, 1971, with steelhead provided from the North Santiam River by the Fish Commission of Oregon. The gametes of each fish were placed into separate polyethylene bags, kept cool over ice in styrofoam boxes, and transported to Corvallis, Oreg. Within 8 hr, the eggs were fertilized and put into Heath incubators.

Approximately one-half the eggs from each mating were placed into one of 240 cells in an incubator, the number of eggs per cell averaging 63. Each cell was made from 2-inch PVC pipe that had been cut to a length corresponding to the distance between the upper and lower screens of an incubator tray. The remaining eggs from each mating were put into 240 cells of a second incubator. Both incubators were provided with chilled, dechlorinated water from a common supply that was maintained at 6.0 ± 0.5 C.

On July 7, the first day that any hatching was observed, approximately 40 eggs were removed from each cell of one incubator and placed into similar cells in a separate system designed for other studies. This system consisted of a series of four interconnected troughs in which the water was circulated by a small pump. The water temperature was maintained at 11 C.

Data included the number of days to hatching for eggs of each group held in an incubator through hatching and the number of days to hatching for eggs of replicate groups held in an incubator for most of the incubation period and in the troughs for the remainder of the period.

Results and discussion — Hatching began in the incubator on July 7 and in the troughs on July 8, and terminated on July 24 and July 17, respectively. Hatching was considered complete when no new hatching was observed for 2 consecutive days. The mean number of days to hatching was 51.9 for eggs in the incubator and 45.4 for eggs in the troughs. The data were submitted to an analysis of variance for a factorial breeding experiment with unequal observations per sib-group (Becker 1967). From the expected mean squares, the variance components attributable to the sources of variation studied were estimated as:

Source		Incubator	Troughs
Males	σ^2_M	0.000(0.003)	0.035(0.013)
Females	σ^2_F	0.019(0.010)	0.047(0.022)
Males × females	σ^2_{MF}	0.126(0.012)	0.110(0.012)
Within cells	σ^2_W	0.290(0.003)	0.408(0.006)

Approximate standard errors are included in parentheses.

From these components, it was then possible to estimate the phenotypic, V_P; additive genetic, V_A; and maternal environment, V_M, components of variance (Table 1).

The differences between the heritabilities estimated from data of the incubator and the troughs, 0.00 and 0.23, respectively, appeared to result from the additive genetic components (Table 1). Although it was not possible to adequately estimate components associated with dominance deviation, V_D; interaction deviation, V_I; and environmental deviation, V_E, the combined estimate for these

TABLE 1. Summary of individual variance components and heritabilities.

Component	Estimated by	Incubator	Troughs
V_P	$(\sigma^2_W + \sigma^2_M + \sigma^2_F + \sigma^2_{MF})$	0.435	0.600
V_A	$4\,(\sigma^2_M)$	0.000	0.140
V_M	$(\sigma^2_F - \sigma^2_M)$	0.019	0.012
$(V_D + V_I + V_E)$	$V_P - (V_A + V_M)$	0.416	0.448
h^2	$\frac{V_A}{V_P}$	0.000	0.230
95% confidence limit for h^2 (Becker 1967)		0.00–0.07	0.06–0.40

values was comparable for the two conditions. Maternal environment effects were only 4 and 2% of total variation for eggs in the incubator and in the troughs, respectively.

Undoubtedly, hatching in the troughs was influenced by the different temperature, light conditions, water quality, and handling procedure to which these eggs were exposed. A genotype–environment interaction was indicated even though the eggs were not transferred to the troughs until after a prolonged exposure to conditions in the incubator. To evaluate this interaction, the two conditions (incubator and trough) were considered as a fixed treatment–effect and an additional analysis for three major sources of variation (treatment, T; males, M; and females, F) was performed on the combined results (Snedecor and Cochran 1967). The genotype–environment interaction was then estimated as the sum of $\sigma^2_{MT} + \sigma^2_{FT} + \sigma^2_{MFT}$ and was equal to 0.089. The relative importance of this value with additive genetic variance ($V_A = 4\sigma^2_M = 0.044$) and nonadditive genetic variance ($V_D = 4\sigma^2_{MF} = 0.260$) indicated that the genetic control of hatching was dependent on the environmental conditions.

These results emphasize the need to exercise caution in extrapolating predictions from experimental results obtained in a single environment. Implicit in these conclusions is the need to consider genotype–environment interactions in the evaluation of the variability in characters that are induced with experimental gradients of environmental factors. Measurements that are characteristic of particular levels of an environmental factor may represent the action of different genes.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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(8) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

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1740. ACKMAN, R. G. 1972. Porous polymer bead packings and formic acid vapor in the GLC of volatile free fatty acids. J. Chromatogr. Sci. 10: 560–565. (8)

1741. FRAIR, W., R. G. ACKMAN, AND N. MROSOUSKY. 1972. Body temperature of *Dermochelys coriacea*: warm turtle from cold water. Science (Washington) 177: 791–793. (8)

Additional FRB Publications

The Fisheries Research Board of Canada also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at Board establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

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Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements de l'Office situés a Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal Fisheries Research Board Canada

Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 2000 P St. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required. Authors are encouraged to submit abstracts in both English and French; those submitted in one language only are translated by the *Journal*.

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and at least 1 mm high when the illustration is reduced to the appropriate size. Drawings should be grouped to facilitate comparisons. Originals must be submitted and should be less than 50 cm in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Photographs should be printed on glossy paper with a wide range in tone. They should be cropped, squared, and mounted on white cardboard, with no spaces between those arranged in groups.

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

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Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal Fisheries Research Board Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 2000 rue P N.-O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries*. L'auteur doit fournir un *résumé*. Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être éxécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscript, il doit spècifier que son institution accepte les frais.

Liste de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral *one*, and between the capital letter O and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal Fisheries Research Board Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (Appendice A). Dactylographiez le résumé sur une page séparée (Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (Appendice C).
Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts;* en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

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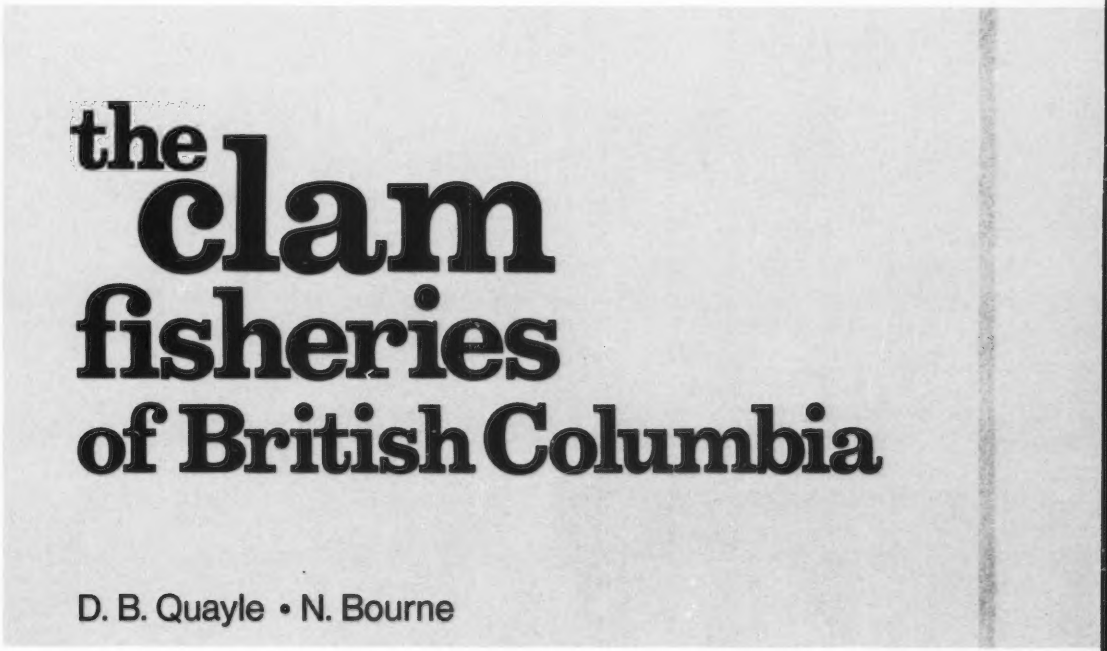

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